EVALUATION OF SERUM ADIPONECTIN LEVELS IN TYPE II DIABETES MELLITUS WITH CHRONIC PERIODONTITIS – AN INTERVENTIONAL STUDY

A Dissertation submitted in
partial fulfilment of the requirements
for the degree of

MASTER OF DENTAL SURGERY

BRANCH – II
PERIODONTOLOGY

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY
Chennai – 600 032

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CERTIFICATE

This is to certify that **Dr. C. KANNAN**, Post Graduate student (2010-2013) in the Department of Periodontics, Tamil Nadu Government Dental College and Hospital, Chennai - 600 003, has done this dissertation titled **"EVALUATION OF SERUM ADIPONECTIN LEVELS IN TYPE II DIABETES MELLITUS WITH CHRONIC PERIODONTITIS – AN INTERVENTIONAL STUDY"** under our direct guidance and supervision in partial fulfillment of the regulations laid down by **The Tamil Nadu Dr. M.G.R. Medical University**, Chennai - 600 032 for **M.D.S.,** (Branch-II) **Periodontics** degree examination.

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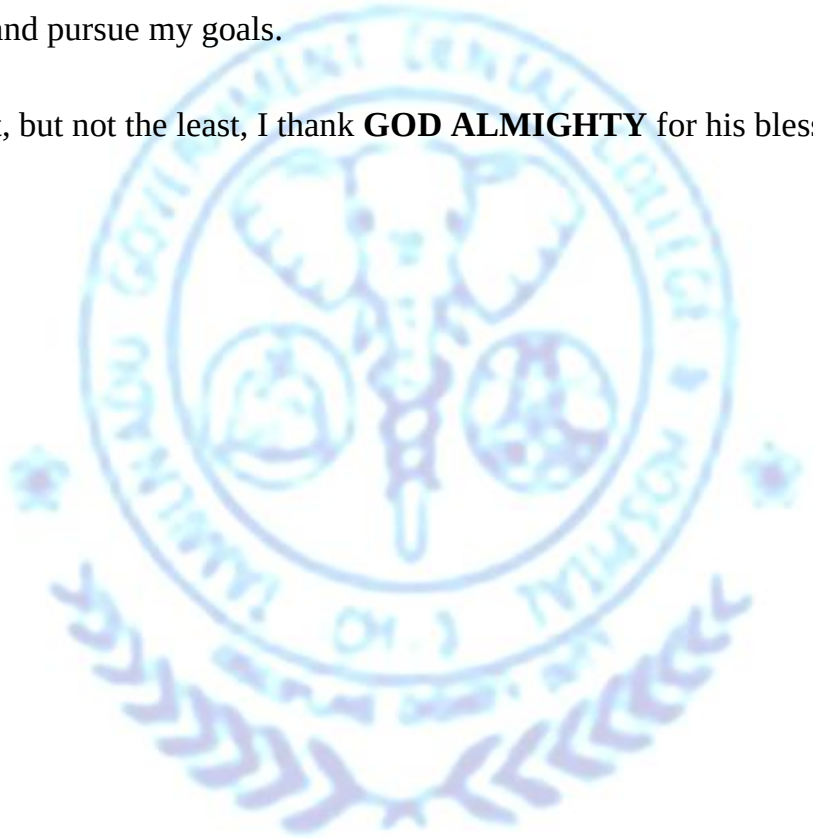
Last, but not the least, I thank **GOD ALMIGHTY** for his blessings.



DECLARATION

TITLE OF DISSERTATION	"Evaluation of serum adiponectin levels in type II diabetes mellitus with chronic periodontitis – An interventional study"
PLACE OF STUDY	Tamil Nadu Government Dental College & Hospital, Chennai-600003
DURATION OF THE COURSE	3 Years
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Mrs. Dr. S. Kalaivani aged 56 years working as **Professor** in Department of Periodontics at the college, having residence address 13th Mother Thersa Street, Srinagar colony, Thirumullaivoyal, Chennai – 62 (herein after referred to as the 'Principal Investigator')

And

Dr. C. Kannan aged 31 years currently studying as **Post Graduate student** in Department of Periodontics, Tamil Nadu Government Dental College and Hospital, Chennai – 600 003, (hereafter referred to as 'the PG student and co-investigator')

Whereas the PG student as part of her curriculum undertakes to research on **"EVALUATION OF SERUM ADIPONECTIN LEVELS IN TYPE II DIABETES MELLITUS WITH CHRONIC PERIODONTITIS – AN INTERVENTIONAL STUDY"** for which purpose the Principal Investigator and the college shall provide the requisite infrastructure based on availability and also provide facility to the PG student as to the extent possible as a Co – investigator

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1.

2.

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Date: 30-11-2011

Title of the Work	Evaluation of Serum adiponectin level in type-II Diabetes Mellitus with Chronic Periodontics – An interventional study
Principal Investigator:	Dr. C. Kannan, II Year P.G.
Department	Dept of Periodontics, Tamil Nadu Government Dental College & Hospital, Chennai-600 003.

The request for an approval from the Institutional Ethical Committee (IEC) was considered for the following on the IEC meeting held on 24.10.2011 at the Principal's Chambers, Tamil Nadu Government Dental College & Hospital, Chennai-3.

"Advise to proceed with the study. Increase number of cases from 40 to 100"

The Members of the Committee, the Secretary and the Chairman are pleased to approve the proposed work mentioned above submitted by the Principal Investigator.

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10. Your work should be carried out under the direct supervision of your Guide/Professor.

30/11/11

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30/11/11

CHAIRMAN

CONTENTS

LIST OF ABBREVIATIONS

AAP	American Academy of Periodontology
BOP	Bleeding on probing
CAL	Clinical Attachment Level
CBC	Complete Blood Count
CEJ	Cemento Enamel Junction
CRP	C-Reactive Protein
DM	Diabetes Mellitus
ELISA	Enzyme Linked Immuno Sorbent Assay
FBS	Fasting Blood Sugar
IL-6	Interleukin-6
IR	Insulin Resistance
kDa	Kilo Dalton
LPS	Lipopolysaccharide
NF-κB	Nuclear Factor kappa B
NSPT	Non Surgical Periodontal Therapy
OPG	Orthopantomogram
PI	Plaque Index
PPD	Pocket Probing Depth
T2DM	Type-2 Diabetes Mellitus
TNF-α	Tumor Necrosis Factor-α

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ABSTRACT

BACKGROUND

Chronic periodontitis is primarily an infection wherein pro-inflammatory cytokines are instrumental in host mediated tissue destructive immune response. Among the cytokines, Tumor necrosis factor-α (TNF-α) has been considered to cause insulin resistance. In type-2 diabetes mellitus, insulin resistance is the main etiological factor which is linked to decreased serum adiponectin level, which has been found recently. Many studies have hypothesized that non-surgical periodontal therapy improves glycemic control and adiponectin level in type-2 diabetes mellitus with chronic periodontitis.

AIM

To evaluate the effect of non-surgical periodontal therapy on serum adiponectin level in Type-II diabetic patients with moderate to severe chronic periodontitis.

METHODS

50 systemically healthy age & gender matched controls with healthy periodontium and 50 type-2 diabetes mellitus patients with moderate to severe chronic periodontitis were enrolled. Venous blood samples were collected at baseline from 100 subjects (Control group and Study group-I) and 3 months after the non-surgical therapy from diabetic patients with moderate to severe chronic periodontitis (Study group-II). Serum level of adiponectin was analysed by Enzyme linked immunosorbent assay (ELISA).

RESULTS

The study group-II showed significant (p=0.000) improvement in clinical periodontal parameters 3 months after the non-surgical therapy. After 3 months, Fasting blood sugar level was significantly (p=0.000) decreased and serum adiponectin level was significantly (p=0.000) increased (8.39µg/mL) in study group-II compared to those in the study group-I (7.52µg/mL).

CONCLUSION

In the present study, type-2 diabetes mellitus patients with moderate to severe chronic periodontitis exhibited improvement compared to baseline value. Non-surgical periodontal therapy improves fasting blood sugar level with elevation of serum adiponectin level in type-2 diabetic patients with moderate to severe chronic periodontitis.

Key words: **Adiponectin, Chronic periodontitis, Type-2 diabetes mellitus**

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by altered glucose tolerance and impaired lipid and carbohydrate metabolism. More than 300 million people worldwide will have diabetes by 2025 and at least 366 million people will have diabetes by 2030[43]. Diabetes mellitus is one among the systemic conditions that can aggravate the progression of chronic periodontitis. Chronic periodontal disease is considered to be the sixth complication of diabetes mellitus[27].

Chronic inflammatory periodontal disease represents a primarily anaerobic gram-negative oral infection that leads to gingival inflammation, destruction of periodontal tissue, loss of alveolar bone and eventually loss of teeth in severe cases. The endotoxin of these micro-organisms induce pro-inflammatory cytokines such as Tumor necrosis factor-α (TNF-α), Interleukin-6 (IL-6) and are instrumental in generating a host-mediated tissue destructive immune response.

Studies[3, 7, 40] have established that adipose tissue is no longer a fatty tissue and it does produce many cytokines, grouply named as 'adipokines'. Among adipokines, adiponectin is a protein hormone or cytokine of 30-KDa complement-related protein (Acrp 30), exclusively produced by adipose tissue[7]. Adiponectin is a insulin-sensitizing hormone with anti-diabetic, anti-inflammatory and anti-atherosclerotic properties[46].

The etiology of type-2 diabetes mellitus is insulin resistance in spite of hyper insulinemia, there is increased hyperglycemia. Increased release and activity of serum C-reactive protein (CRP) and Tumor necrosis factor-α (TNF-α) are mainly considered to be responsible for the development and progression of insulin resistance in type-2 diabetes mellitus. Adiponectin has been thought to be associated with insulin

resistance[21] and plays an important negative regulatory role in some physiological and pathological processes[53]. Few studies[8,22,36,50] have been done regarding initial periodontal treatment for type-2 diabetes patients with moderate to severe chronic periodontitis to evaluate the impact on pro-inflammatory cytokines, glycemic control and serum adiponectin level.

Hence, the present study was undertaken to evaluate the effects of non-surgical periodontal therapy on serum adiponectin levels in type-II diabetes mellitus patients with moderate to severe chronic periodontitis.

AIM AND OBJECTIVES

The aim of the study was to evaluate the effect of non-surgical periodontal therapy on serum adiponectin level in Type-II diabetic patients with moderate to severe chronic periodontitis.

For this purpose the following objectives were undertaken:

1. To estimate the level of adiponectin in type-II diabetic patients with moderate to severe chronic periodontitis.
2. To investigate the effect of non-surgical therapy on serum adiponectin level as a marker of insulin resistance.
3. To correlate and compare the serum adiponectin level with blood sugar level and periodontal status of type-II diabetes mellitus patients with moderate to severe chronic periodontitis before and after treatment.

REVIEW OF LITERATURE

ADIPONECTIN

HISTORICAL BACKGROUND AND GENERAL PROPERTIES

In 1992, Grimshaw et al[14]**,** stated that mice fed with omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have shown increased plasma adiponectin levels. Furthermore, Berberine is an herbal folk medicine, which also has been shown to increase adiponectin expression which partly explains its beneficial effects on metabolic disturbances.

Lodish et al. **1995**[26] identified a secretory protein from murine 3T3-L1 adipocytes and named it adipocyte complement-related protein of 30 kDa (Acrp30). It is a structural homolog to complement factor C1q and to a hibernation-specific protein isolated from the plasma of Siberian chipmunks. It forms large homo-oligomers that undergo a series of post-translational modifications.

Scherer PE et al. 1995[40] observed that the adiponectin is a 147 amino acid protein and consisits of four distinct regions. The first is a short signal sequence that targets the hormone for secretion outside the cell; next is a short region that varies between species; the third is a 65-amino acid region with similarity to collagenous proteins; the last is a globular domain. Overall this gene shows similarity to the complement 1q factors (C1q). It possesses a short N-terminal variable region followed by several collagen repeats (Gly-X-Y) and finally a large C-terminal globular domain.

Maeda K et al. 1996[30] assessed the human adiponectin gene which was cloned through systematic sequencing of an adipose-tissue library. The apM1 gene encodes a 244 amino acid open reading frame, containing a putative signal sequence

repeat (66 amino acids) followed by a cluster of aromatic residues near the C terminus having high local resemblance to collagens X and VIII and complement factor C1q.

Based on SDS-PAGE1 and crystallographic studies, structure of adiponectin appears to form a variety of higher order structures. Also **Shapiro L et al. 1998**[41] demonstrated that the adiponectin monomers assemble into homotrimers with the three globular domains adjacent to one another and the three collagen-like regions forming a collagen triple helix. These trimers then assemble into hexamers and other high molecular weight (HMW) complexes.

In 1999, a group at Osaka University[3] isolated the human adipose-specific transcript, the apM1 gene product, which was found to be a soluble matrix protein, and named it adiponectin. It was identified as a distinct protein among the adipokines because the plasma concentration of adiponectin decreases upon accumulation of visceral fat.

In 2003, a group from Japan[51] isolated complementary DNAs encoding the adiponectin receptors 1 and 2(AdipoR1 and AdipoR2) by expression cloning. These two adiponectin receptors have seven-transmembrane domains, but they are distinct from the topology of G-protein-coupled receptors. The AdipoR1 gene encodes for a 375-amino-acid protein with an estimated molecular mass of 42.4 kDa, whereas AdipoR2 encodes for a 311-amino-acid protein of 35.4 kDa.

Waki et al. 2003[47] described that the various adiponectin species affect liver hepatocytes and skeletal muscle myocytes differently. Only the high molecular weight (HMW) multimer and the hexamer forms of adiponectin act on hepatocytes via AMP-activated protein kinase (AMPK) to inhibit glucose production and reduce intracellular triglycerides (TGs) and insulin resistance. By contrast, the Cys39ser mutant trimer and globular domain mutants, as well as all wild-type adiponectin

species, act on myocytes via AMPK to stimulate glucose uptake and reduce intracellular TGs and insulin resistance.

Wang J et al. 2004[48] revealed that the T-cadherin is an adiponectin-binding protein through subsequent DNA analysis. T-cadherin is a unique cadherin molecule that lacks the transmembrane and cytoplasmic domains and is bound to the surface membrane through a glycosylphosphatidylinositol (GPI) anchor and it can bind to the hexameric and HMW forms of adiponectin but not to monomer globular and trimeric forms. T-cadherin is ubiquitously expressed, with the highest expression found in the heart and the aortic, carotid, iliac, and kidney arteries and it is critical for the association of adiponectin protection against cardiac stress in mice.

Nedvídkova J et al. 2005[34] showed that the gene was localized to chromosome 3q27, a region highlighted as affecting genetic susceptibility to type-2 diabetes and obesity. The gene was investigated for variants that predispose to type–2 diabetes. Several single nucleotide polymorphisms in the coding region and surrounding sequence were identified from several different populations, with varying prevalence, degrees of association and strength of effect on type-2 diabetes.

Choi BH et al. 2009[7] revealed that the adiponectin (also known as GBP28, apM1, Acrp30, or AdipoQ) is a 244-residue protein that is produced largely by white adipose tissue (WAT). Adiponectin has structural homology with collagens VIII and X and complement factor C1q, and circulates in the blood in relatively large amounts in different molecular forms. Furthermore, adiponectin circulates in the bloodstream in trimeric, hexameric, and high-molecular-mass species, while different forms of adiponectin have been found to play distinct roles in the balance of energy homoeostasis

ASSOCIATION BETWEEN ADIPONECTIN AND TYPE-2 DIABETES MELLITUS, PERIODONTITIS

Uysal et al. 1997[46] observed that the adiponectin mainly affects insulin receptors which are a part of insulin sensitivity cascade rather than HbA1c level and plays an important negative regulatory role in some physiological and pathological processes including multiple protective roles such as anti-diabetic, anti-atherosclerotic and anti-inflammatory factors. It regulates glucose and lipid metabolism, improves insulin sensitivity, reduces hepatic glucose production and also they stated that the hypoadiponectinemia is correlated with increased insulin resistance during the development Type-2 Diabetes mellitus.

Hotta et al. 2000[16] reported that adiponectin is a novel, adipose-specific protein abundantly present in the circulation, and it has anti-atherogenic properties. Plasma levels of adiponectin in the diabetic subjects without CAD were lower than those in nondiabetic subjects. The plasma adiponectin concentrations of diabetic patients with CAD were lower than those of diabetic patients without CAD. Significant, univariate, inverse correlations were observed between adiponectin levels and fasting plasma insulin ($r=-0.18$, $P<0.01$) and glucose ($r=-0.26$, $P<0.001$) levels. These results suggested that the decreased plasma adiponectin concentrations in type-2 diabetes patients compared with non-diabetic.

Imagawa A et al. 2002[19] stated that the serum adiponectin levels are elevated in type I diabetic patients (ie: patients with reduced levels of circulating insulin) as well as in patients with genetically defective insulin receptors when compared with healthy controls. Furthermore, hyperinsulinaemia significantly lowers plasma adiponectin levels under euglycaemic conditions. In addition, the HMW form of

adiponectin is selectively down-regulated in hyperinsulinaemia and type II diabetes. It is possible that insulin may activate some signaling pathways that indirectly suppress adiponectin biosynthesis and secretion.

Yamauchi T et al. 2002[52] confirmed that adiponectin (Ad) is a hormone secreted by adipocytes that regulates energy homeostasis and glucose and lipid metabolism. Further, they showed that phosphorylation and activation of the 5'-AMP-activated protein kinase (AMPK) are stimulated with globular and full-length Ad in skeletal muscle and only with full-length Ad in the liver. In parallel with its activation of AMPK, Ad stimulates phosphorylation of acetyl coenzyme A carboxylase (ACC), glucose uptake and reduction of molecules involved in gluconeogenesis in the liver. Blocking AMPK activation by dominant-negative mutant inhibits each of these effects, indicating that stimulation of glucose utilization and fatty-acid oxidation by Ad occurs through activation of AMPK.

He W et al. 2003[15] identified Peroxisome Proliferator- Activated Receptors- γ (PPARγ) is a member of the PPAR subfamilies of transcription factors, which is expressed mainly in adipose tissue and which is considered to be a positive regulator of adiponectin gene expression. Targeted deletion of PPARγ in adipose tissue of mice results in marked adipocyte hypocellularity and hypertrophy, elevated levels of plasma free fatty acids and triglyceride, and decreased levels of adiponectin. Furthermore, PPARγ increases adiponectin levels and secretion by stimulating the expression of proteins involved in adiponectin assembly.

Kadowaki et al. 2006[21] reported that adiponectin, the major adipocyte secretory protein, has been thought to be associated with insulin resistance (IR) and hypoadiponectinemia, caused by interactions of genetic factors such as SNPs in the Adiponectin gene and environmental factors causing obesity, appears to play an

important causal role in insulin resistance, type 2 diabetes, and the metabolic syndrome, which are linked to obesity. The adiponectin receptors, AdipoR1 and AdipoR2, which mediate the antidiabetic metabolic actions of adiponectin, have been cloned and are downregulated in obesity-linked insulin resistance. Upregulation of adiponectin is a partial cause of the insulin-sensitizing and antidiabetic actions of thiazolidinediones.

Lu HL et al. 2006[29] showed that plasma adiponectin was associated with the disorder of metabolism of glucose and lipid in diabetes. The levels of plasma adiponectin was correlated negatively with BMI, blood glucose, insulin resistance index and triglyceride (respectively, r=-0.55, P<0.01; r=-0.51, P<0.05; r=-0.52, P<0.05; r=-0.39, P<0.05), while it was positively correlated with insulin sensitive index (r=0.45, P<0.05). They concluded that the relationship between adiponectin hormone and insulin sensitivity suggests that it may take part in the development of insulin resistance of type 2 diabetes and levels appear to correlate negatively with insulin sensitivity.

Yamaguchi N et al. 2007[53] explored the role of adiponectin in the etiology of periodontitis using the D clone of RAW264, a clone that exhibits highly efficient osteoclast formation, to determine whether adiponectin acts as a regulatory molecule in osteoclast formation stimulated by lipopolysaccharide of periodontopathic bacteria. Further, they observed that adiponectin acted as a potent inhibitor of osteoclast formation stimulated by Toll-like receptor-4 (TLR4) ligand and receptor activator of NF-κB ligand (RANKL). These results strongly suggested that adiponectin may function as a negative regulator of lipopolysaccharide/RANKL-mediated osteoclast formation in periodontal disease.

Kopp A et al. 2010[24] investigated that the mechanisms of Toll-like receptor (TLR)-induced prodiabetic and proinflammatory activation of adipocytes. They concluded that macrophage activating lipopeptide-2 (MALP-2) is able to induce IL-6 and monocyte chemoattractant protein-1 (MCP-1) release in adipocytes isolated from inflamed adipose tissue, whereas these adipocytes lost their ability to respond to LPS. The present results shows a role of the adipose tissue in innate immunity and TLR-ligand induced proinflammatory and prodiabetic activation of adipocytes might couple visceral adipose tissue dysfunction with insulin resistance and type 2 diabetes mellitus.

Reiko Furugen et al. 2010[39] stated that pro-inflammatory cytokines such as TNF-α and IL-6 are induced after stimulation with lipopolysaccharide (LPS) of gram-negative periodontopathic bacteria. These cytokines increased by the progression to severe forms of periodontitis may affect glucose metabolism. Hence, the elevation of these cytokines attributable to periodontitis could increase the risk for insulin resistance and suppress the adiponectin production leads to reduce insulin sensitivity and worsen glycemic control.

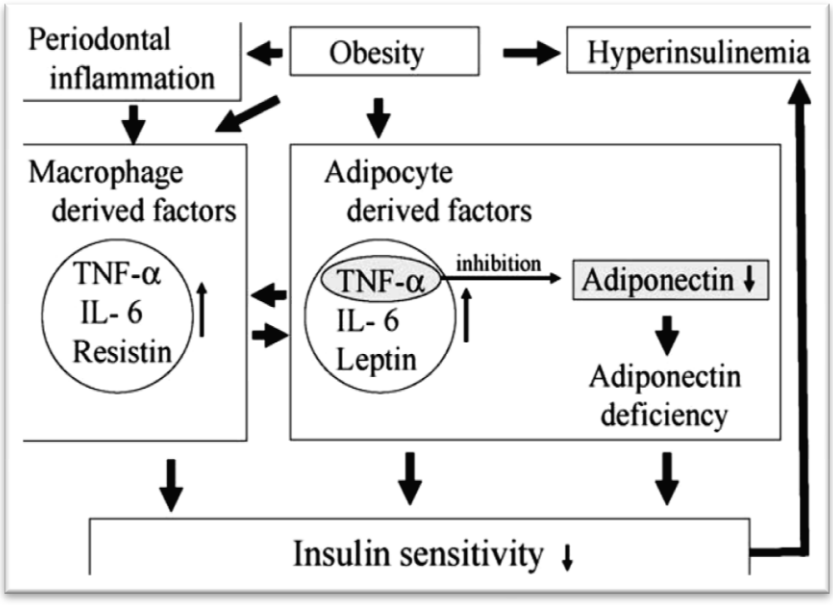


Figure 1: T**he relationship among periodontitis and adipokines**

Zhang X et al. 2011[54] stated that on a high-fat diet (HFD), the ap2-dn-JNK mice displayed a marked suppression of both JNK1 and JNK2 activation in their adipose tissue, accompanied by a marked reduction in weight gain, fat mass, and size of the adipocytes. The transgenic mice were resistant to the deleterious impact of an HFD on systemic insulin sensitivity, glucose tolerance. These changes were accompanied by reduced macrophage infiltration in adipose tissue, decreased production of proinflammatory adipokines and increased expression of adiponectin. These results concluded that the selective suppression of JNK activation in adipose tissue leads to decreased systemic inflammation and increased serum adiponectin level.

Ioanna Xynogala et al. 2012[20] showed that the serum levels of adiponectin were increased in insulin treated diabetic rats in the presence of periodontitis, while serum IL-6 levels did not change. Furthermore, adiponectin levels were statistically significantly higher at the end of the experiment compared with levels on day 16 in the periodontitis group ($p<0.01$), but did not change in insulin treated diabetic rats without periodontitis.

Dominik Kraus et al. 2012[10] revealed that adiponectin modulates critical effects of lipopolysaccharide (LPS) from P.gingivalis on oral epithelial cells (OECs). Gingival tissue sections showed a strong synthesis of adiponectin and its receptors in the epithelial layer. Adiponectin abrogated significantly the stimulatory effect of LPS. Similarly, it inhibited significantly the LPS-induced decrease in cell viability and increase in cell proliferation and differentiation. Also, adiponectin led to a time-dependent induction of the anti-inflammatory mediators IL-10 and hemeoxygenase-1 and blocked the LPS-stimulated Nuclear Factor-κB nuclear translocation.

ASSOCIATION BETWEEN PERIODONTITIS AND TYPE-2 DIABETES MELLITUS

Pociot F et al. 1993[37] stated that the circulating monocytes from diabetic patients exhibit an exaggerated inflammatory response to gram-negative bacterial lipopolysaccharides, releasing large amounts of inflammatory mediators and pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α. This hyper responsive monocytic phenotype is not associated with hyperglycemia and potential predisposition to tissue breakdown.

Weidman E et al. 1996[49] reported that individuals with sustained hyperglycemia, proteins become irreversibly glycated to form advanced glycation end products (AGEs). These stable carbohydrate-containing proteins have multiple effects on cell-to-cell and cell-to-matrix interactions and are commonly thought to be a major link between the various diabetic complications. The formation of AGEs also occurs in the periodontium and higher levels of periodontal AGE accumulation and periodontal destruction are found in those with diabetes than in non-diabetic subjects.

Loesche WJ et al. 1998[28] stated severe chronic periodontitis represents a sub-clinical septicemic state. It can produce some inflammatory cytokines (e.g. CRP, TNF-α and IL-6) in the local tissue, as well as elevating their circulating levels. CRP is an important mediator of inflammation, mainly synthesized in the liver. TNF-α is another important inflammatory cytokine, closely linked to insulin resistance, which plays a role in the regulation of CRP expression and both the levels are increased in Type – 2 Diabetes patients with periodontitis.

Qi C et al. 2000[38] reported that the elevated levels of TNF-α alter intracellular insulin signaling (inhibiting tyrosine kinase activity of insulin receptor) and reduce

the synthesis of insulin responsive glucose transporter, creating an insulin resistance syndrome similar to insulin resistance that characterizes type-2 diabetes.

Hujoel PP et al. 2001[18] described that the dentogingival surface area (DGES) comprises both the sulcular and junctional epithelium, present in health, as well as any intervening pocket epithelium present in periodontitis. Individuals without periodontitis had a typical DGES of 5 cm^2. Among individuals with periodontitis, the mean DGES in the three samples ranged from 8 cm^2 (ranging from 1 to 29 cm^2) to 20 cm^2 (ranging from 2 to 44 cm^2). It was concluded that the mean DGES among individuals with periodontitis or the cumulative surface area of ulcerated pocket epithelium has been estimated to ranges from 8 to 20 cm^2, which is approximately the size of the palm of an adult hand.

Arner P et al. 2005[4] confirmed that the periodontal treatment not only reduces clinically evident inflammation, but also improves the glycemic control and reduces insulin resistance (IR) and improves β-cell function in type-2 diabetes mellitus (T2DM) patients. These findings indicate that inflammation is involved in the pathogenesis of IR and T2DM, which is regarded as key processes in the mechanism of T2DM. Furthermore, IR plays a central role in the development of T2DM.

Nishimura et al. 2005[35] found that chronic periodontal inflammation can lead to increased serum levels of TNF-α, thus inducing the phosphorylation of serine residues in the insulin receptor substrate-1, prompting the target cells to produce insulin resistance (IR). TNF-α and other inflammatory mediators may activate the intracellular pathways, such as the I-kappa-B (IκB), I-kappa-B kinase-β (IκKβ), nuclear factor-kappa B (NF-κβ) and the protein c-Jun N-terminal Kinase (JNK) axes, amplify and aggravate low-grade inflammation, and these processes may become self-

perpetuating through a positive feedback loop created by the pro-inflammatory cytokines and lead to IR and diabetes.

Engebretson et al. 2007[11] suggested that the elevated circulating tumour necrosis factor-alpha (TNF-α) may contribute to insulin resistance in patients with type 2 diabetes. The source of plasma TNF-α has been thought to be adipocytes associated with obesity, but inflammation and infection result in TNF-α production as well. Furthermore, chronic periodontitis is associated with plasma TNF-α levels in subjects with type-2 diabetes supports the hypothesis that periodontal infection and inflammation may contribute to insulin resistance. TNF-α showed a significant positive correlation with attachment loss ($r=0.40$, $p=0.009$), plasma endotoxin ($r=0.33$, $p=0.03$). A dose-response relationship was observed between periodontitis severity and TNF-α ($p=0.012$)

Mealey BL et al. 2008[32] evaluated that chronic systemic inflammatory state induced by periodontal disease which may contribute to insulin resistance through a "feed-forward" mechanism that worsens glycemic control. There was a dose-response relationship between the severity of PD and serum TNF-α level, which suggested that periodontal disease may play a major role in elevating levels of this cytokine, which is closely linked to insulin resistance and thereby aggravate metabolic control.

Lei Chen et al. 2010[25] stated chronic periodontitis was associated with glycemic metabolic and serum hs-CRP levels in patients with type-2 diabetes. The results revealed that an increased mean PD had significantly higher levels of HbA1c and hs-CRP ($P<0.05$) and no significant difference was found among different groups in the levels of serum TNF-α , fasting glucose and lipid profiles but, after controlling for age, gender, body mass index, duration of diabetes mellitus, smoking, regular physical exercise and alcohol consumption positive correlations were found between

mean PD and HbA1c (r=0.2272; P=0.009) and between mean PD and hs-CRP (r=0.2336; P=0.007).

EFFECT OF PERIODONTAL TREATMENT ON SERUM ADIPONECTIN AND BLOOD GLUCOSE LEVEL

Miller LS et al. 1992[33] demonstrated that combination of scaling and root planing with systemic doxycycline therapy is associated with an improvement in periodontal status that is accompanied by significant improvement in glycemic control, as measured by the glycated hemoglobin assay (HbA1c) as well as fasting plasma glucose level (FPG).

Stewart JE et al. 2001[44] reported that significant improvement in glycemic control after treatment. After periodontal therapy, the weighted average decrease in absolute HbA1c values was 0.4%, but this was not found to be statistically significant. The addition of adjunctive systemic antibiotics to the mechanical therapy regimen resulted in an average absolute reduction of 0.7%. Again, this reduction did not achieve a level of statistical significance.

Kiran et al. 2005[23] showed that non-surgical periodontal treatment is associated with improved glycemic control in type-2 diabetes patients. After SRP, poorly controlled diabetes patients were reported to exhibit significant reductions in glycated haemoglobin (HbA1c) and fasting plasma glucose levels at the 3-month time point compared to levels at baseline and 1-month and also exhibit significant reductions in PD, PI, BOP and CAL. However, the baseline and 1-month HbA1c levels were indifferent for these clinical parameters.

Furugen R et al. 2008[12] showed that elderly Japanese people with periodontitis (at least one tooth that displayed a probing pocket depth of >6 mm) was

significantly associated with higher serum resistin levels and total leukocyte counts. No significant differences were observed in adiponectin, IL-6 and TNF-α levels between subjects with and without periodontitis, but serum adiponectin tended to decrease in patients with periodontitis.

Debora C et al. 2009[9] demonstrated that the periodontal therapy improved glycemic control in patients with type-2 diabetes. However, the reduction in HbA1c values for the group who received full mouth scaling and root planning, was statistically significant, but not for the group who received full mouth scaling and root planning and antibiotics. The changes in fasting glucose levels were not significant for either group. However, the reduction in HbA1c values reached statistical significance only in the group received scaling and root planing alone.

Matsumoto et al. 2009[31] revealed that anti-microbial periodontal treatment (APT) and periodontal maintenance (PM) not only improve periodontal disease but also increase serum adiponectin in T2DM patients. Test group received scaling with ultrasonic devices at baseline and APT biweekly for 2 months while control group received scaling at baseline and mechanical tooth cleaning (MPT) at the same interval. At 6 months, all patients received mechanical tooth cleaning as PM. Adiponectin concentrations in test group had significantly increased by 31.4% after APT ($p=0.024$) and by 30.4% after PM ($p=0.002$) compared with baseline. The percentage of ≥4 mm probing depths (PD) had shown 8.3% and 9.3% reduction after APT and PM ($p=0.046$, 0.02) in test group while 5.0% reduction after MPT in control group.

Correa FOB et al. 2010[8] reported that clinically successful non-surgical periodontal therapy tended to reduce systemic inflammation and the concentration of

some circulating cytokines. Further, the results showed that all clinical parameters (plaque index, gingival bleeding index, bleeding on probing, probing depth and clinical attachment level) were significantly improved 3-months after the periodontal therapy. A univariate comparison suggested a tendency towards a decrease of the measured biomarkers, most pronounced for TNF-α and FIB, after therapy. Periodontal treatment also reduced fasting plasma glucose and hs-CRP levels, albeit not significantly.

Kardesler L et al. 2010[22] demonstrated that the patients with type 2 diabetes and chronic periodontitis exhibited similar clinical periodontal improvements as their systemically healthy counterparts. Initial periodontal treatment appeared to decrease fasting plasma glucose (FPG) and HbA1c level, thereby improve glycemic control in poorly controlled patients with diabetes. Decreases in levels of IL-6, TNF-α, CRP and leptin and an increase in adiponectin levels after periodontal therapy may be a function of glycemic control in patients with type-2 diabetes.

Pariksha Bharti et al. 2011[36] stated that periodontal treatment improves periodontal status and glycemic control with elevation of serum adiponectin in type-2 diabetes patients. The results suggested that HbA1c is reduced by amelioration of insulin resistance due to elevated serum adiponectin after periodontal treatment and improvements of PPD and BOP also were observed. Generalized linear model revealed that changes of serum adiponectin and TNF-α and change of BOP correlated significantly with the reduction of HbA1c at 6 months after periodontal treatment.

Wei-Lian Sun et al. 2011[50] showed that periodontal intervention can improve glycemic control, lipid profile and IR, reduce serum inflammatory cytokine levels and increase serum adiponectin levels in moderately to poorly controlled T2DM patients. The levels of clinical periodontal variables, the probing depth, attachment loss,

bleeding index, and plaque index were improved significantly in T2DM-Treated group after 3 months compared to T2DM-Non treated group (all $p<0.01$). After 3 months, the serum levels of hs-CRP, TNF-α, IL-6, fasting plasma glucose (FPG), glycosylated hemoglobin (HbA1c), fasting insulin (FINS) and homeostasis model of assessment - insulin resistance (HOMA-IR) significantly decreased and adiponectin was significantly increased in T2DM-Treated group compared to those in the T2DM-Non treated group ($p<0.05$ or $p<0.01$).

MATERIALS AND METHODS

STUDY DESIGN AND SUBJECT SELECTION:

The study was approved by the Institutional Ethical Committee. About 100 subjects in the age group of 35-60 years who attended the outpatient section of Department of Periodontics, Tamil Nadu Government Dental College, Chennai participated in the study. The patients were divided into 3 Groups, Control group - 50 subjects with healthy periodontium and Study group - 50 type-2 diabetes mellitus subjects with moderate to severe chronic periodontitis, the same study group before treatment considered as Study group-I and after treatment considered as Study group-II.

INCLUSION CRITERIA:

- Patients willing for voluntary participation and signing the informed consent.
- Age : 35-60 years
- Gender : Both males and females
- **Healthy subjects: (CONTROL GROUP)**

a. Probing depth <3mm

b. Less than 20% of sites with gingival bleeding

c. Fasting blood sugar level 80-110 mg/dl

d. Good oral hygiene status with plaque index score less than 1

e. Absence of Clinical Attachment Loss as determined by CAL (Clinical Attachment Level) measurements i.e. CAL=0

- **Type-II Diabetes mellitus with moderate to severe generalized chronic periodontitis: (STUDY GOUP-I)**

a. To obtain physician opinion prior to non surgical periodontal therapy.

b. No change in courses of treatment

i.e. there was no change in oral anti-diabetic drug 3 months before and during the study.

c. Patients having at least 20 teeth

d. ≥ 2 teeth per quadrant, excluding third molars with Probing Pocket Depth ≥5mm.

e. Presence of Clinical Attachment Loss as determined by CAL measurements ≥3mm^2.

f. Patients having greater than 126mg/dl of fasting blood glucose level

g. Radiographic evidence of alveolar bone loss

EXCLUSION CRITERIA:

- History of Smoking or use of tobacco in any forms
- History of any systemic diseases except Diabetes Mellitus (Hypertension, Cardiovascular diseases and Kidney, Liver or Lung diseases)
- Patients with a history of periodontal treatment in the past 6 months
- History or presence of any other chronic infectious diseases

- Pregnancy and lactation
- Alcohol consumption

STUDY PROTOCOL:

1. Institutional Ethical Committee approval.
2. Medical history and Informed consent.
3. Periodontal examination using clinical parameters namely, Gingival Bleeding Index, Plaque Index, Probing Pocket Depth and Clinical Attachment Level.
4. Orthopantomogram (OPG) for radiographic evaluation of generalized chronic periodontitis.
5. Complete blood count (CBC)
6. Estimation of Fasting blood glucose (FBS) level by Glucometer.
7. Collection of blood samples.
8. Estimation of Adiponectin level in serum by ELISA method.

Following selection of subjects, written informed consent was obtained after explaining the study procedure. Examination was preceded by a thorough medical and dental history of the subjects. Intra-oral examination was done using Mouth mirror and William's periodontal probe. Periodontal evaluation was done by measuring Gingival Bleeding Index (GBI), Plaque Index (PI), Probing Pocket Depth (PPD), Clinical Attachment Level (CAL) and Orthopantomogram (OPG).

ARMAMENTARIUM

CLINICAL EXAMINATION AND SAMPLE COLLECTION:

Mouth mirror

William's Periodontal Probe

Tweezer

Head cap

Surgical gloves

Face mask

Spirit

Sterile cotton

Torniquette

5ml disposable syringe with 23 gauge disposable needle

Vaccutainer tubes

Centrifuge machine

Micropipette

Eppendorf tubes

Thermos box with gel pack

One-touch Horizon Glucometer

FOR NON-SURGICAL PERIODONTAL THERAPY:

Mouth mirror

Explorer

Tweezer

Scalers and Curettes

Kidney Tray

Cotton Rolls

Disposable Gloves

Disposable Facemask

Disposable Headcap

Disposable syringe

Local Anaesthetic solution

SAMPLE STORAGE:

-20 ˚C Freezer

ELISA PROCEDURE:

Test tubes

Eppendorf tubes

Plastic rack

Autoclaved plastic pipette tips

Micropipette

ELISA washer

ELISA reader

PERIODONTAL EXAMINATION

CLINICAL PARAMETERS:

GINGIVAL BLEEDING INDEX[1]:

Starting distobuccally, the probe was inserted slightly into the sulcus and run to the buccal and mesial surfaces of every tooth at an angle of about 45'. This was repeated for all teeth present. Probing was similarly carried out at palatal/lingual sites. Any gingival units that exhibited bleeding were recorded. The total number of bleeding sites per tooth was recorded for every tooth except the third molars.

Criteria for scoring:

Positive score (+) - Presence of bleeding within 10 seconds

Negative score (-) - Absence of bleeding

$$\% \text{ of bleeding sites} = \frac{\text{Total number of positive score}}{\text{Total number of surfaces of all teeth}} \times 100$$

PLAQUE INDEX[42] :

All teeth were examined at 4 surfaces (disto-facial, facial, mesio-facial, lingual/palatal) and were scored as follows

Criteria for scoring:

Score 0	No plaque
Score 1	Plaque not visible to the naked eye, detected by explorer
Score 2	Thin to moderate accumulation of soft deposits within the gingival pocket or the tooth and gingival margin, visible to the naked eye
Score 3	Abundance of soft matter within gingival pocket or on tooth surface and gingival margins, inter-dental area stuffed with soft debris

Calculation:

Plaque index for the tooth = Total score from 4 areas / 4

Plaque index for the individual = Total plaque indices for all teeth /

No. of teeth examined.

Interpretation:

Score 0 - Excellent oral hygiene

0.1 to 0.9 - Good oral hygiene

1.0 to 1.9 - Fair oral hygiene

2.0 to 3.0 - Poor oral hygiene

PROBING POCKET DEPTH (PPD)[13, 6] (mm):

Probing Pocket Depth was measured from the gingival margin to the base of the pocket using William's Periodontal Probe. The probe was walked within the gingival sulcus along the circumference of the tooth. Six measurements were made per tooth

- Mesiobuccal, Midbuccal,

  Distobuccal, Mesiolingual,

  Midlingual, Distolingual.

CLINICAL ATTACHMENT LEVEL (CAL)[6] (mm):

- Clinical Attachment Level was measurement from the Cemento – enamel junction (CEJ) to the base of the pocket in millimeters using William's Periodontal Probe. Three measurements were made on the buccal aspect and three on the lingual aspect of each tooth – total of six sites per tooth such as Mesiobuccal, Midbuccal, Distobuccal, Mesiolingual, Midlingual, and Distolingual sites.
- When the gingival margin was located on the anatomic crown, the level of the attachment was determined by subtracting from the probing pocket depth, the distance from the gingival margin to the Cemento-Enamel Junction (CEJ). If both were the same, the loss of attachment was calculated to be Zero.
- When the gingival margin coincided with the CEJ, the loss of attachment was calculated as equaling the probing pocket depth.
- When the gingival margin was located apical to the CEJ, the loss of attachment was greater than the probing pocket depth and therefore the distance between the CEJ and the gingival margin were added to the PPD.

FASTING BLOOD SUGAR ESTIMATION:

After cleaning of the middle finger of the left hand for each patient using spirit and lancet, a drop of blood was obtained and fasting blood glucose level was estimated using One-touch Horizon glucometer and recorded.

BLOOD SAMPLE COLLECTION FOR CONTROL AND STUDY GROUP-1 (PRE-TREATMENT) SUBJECTS:

After skin preparation, 5ml of fasting venous blood sample was obtained from each patient by using disposable hypodermic syringe with 23 gauge needle by venipuncture without stasis from antecubital fossa between 8.00 to 10.00 AM and transferred to a plain vaccutainer tube. The blood sample was allowed to clot for 30 minutes in the vaccutainer tube and then centrifuged for 15 minutes at 3000 rpm to separate the serum. Then 500µL of serum sample was divided in aliquots and stored at -20˚C until analysis.

BLOOD SAMPLE COLLECTION FOR STUDY GROUP - 11 (POST-TREATMENT) SUBJECTS:

Non-surgical periodontal therapy (Scaling and Root planing) was performed and the patient was instructed to maintain oral hygiene. After 3 months, periodontal status were re-evaluated and blood samples obtained same as previously mentioned and then centrifuged for 15 minutes at 3000 rpm to separate the serum. Then 500µL of serum sample was divided in aliquots and stored at -20˚C until analysis.

PROCEDURE FOR ADIPONECTIN ANALYSIS:

1. ELISA METHOD:

The circulating adiponectin, is expressed and secreted exclusively by adipose tissue and is measurable in serum by ELISA.

In this study Orgenium Human Adiponectin, R&D Laborateries ELISA Kit, FINLAND was used.

Contents of ELISA kit:

- **Adiponectin Microplate (Item A) :** 1 plate

  96 well polystyrene microplate coated with anti-human Adiponectin.

- **Wash Buffer Concentrate (20x) (Item B) :**

  25ml of 20x concentrated solution.

- **Adiponectin Standards (Item C) :** 2 vials

  80 ng of recombinant adiponectin in a buffered protein base added with preservatives.

- **Assay Diluent A (Item D) :** 2 bottles

  30ml of diluents buffer, 0.09% sodium azide as preservative.

- **Assay Diluent C (Item L) :** 2 bottles

  15ml of diluents buffer

- **Assay Diluent B (Item E) :**

  15ml of 5x concentrated buffer

- **Detection Antibody Adiponectin (Item F) :** 2 vials

  Biotinylated anti-human Adiponectin (each vial is enough to assay half microplate).

- **HRP-Streptavidin Concentrate (Item G) :**

  8 µl 4000x concentrated HRP-conjugated streptavidin.

- **TMB one-step color Substrate Reagent (Item H) :**

12ml of 3,3' ,5,5'- tetramethylbenzidine (TMB) in buffer solution.

- **Stop solution (Item I) :** 1 vial

  8 mL of 2 M sulfuric acid.

REAGENT PREPARATION:

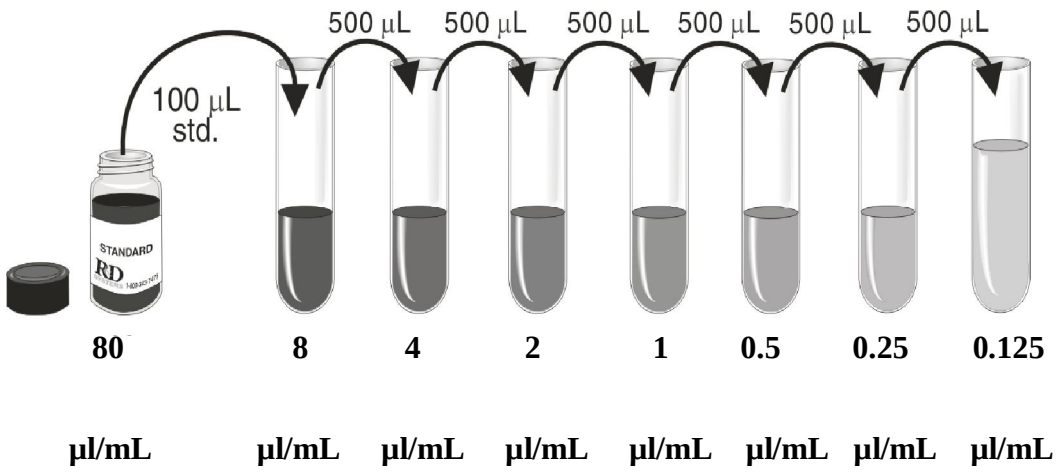
1. All reagents and samples allowed to attain room temperature (18- 25˚C).
2. Sample dilution :

   A 200 – fold dilution of serum sample was done - 2µl sample + 398 µl Assay Diluent A (Item D).
3. Assay Diluent B should be diluted 5 – fold with deionized or distilled water.
4. Preparation of standard :

   The vial of Item C was briefly spined and then added 800 µl Assay Diluent A or Assay Diluent C into Item C vial, a 50 µg/ml standard prepared. The powder thoroughly mixed and dissolved. Added 180 µl Adiponectin standard (50 µg/ml) from the vial of Item C, into a tube with 320 µl Assay Diluent A or Assay Diluent C, a 80 µg/ml stock standard solution prepared. The solution was Pipetted 500 µl Assay Diluent A or Assay Diluent C into each tube. The stock standard solution used to produce a dilution series. Each tube mixed thoroughly before the next transfer. Assay Diluent A or Assay Diluent C served as the Zero standard (0 µg/ml).
5. The Detection Antibody vial (Item F) was briefly spined before use. Then added 100µl of 1 x Assay Diluent B into the vial, a detection antibody concentrate prepared. Pipetted up and down and gently mixed. The detection antibody concentrate was diluted 80 – fold with 1 x Assay Diluent B and used in step 4 of Part VI Assay Procedure.

6. The HRP-Streptavidin concentrate vial (Item G) was briefly spined and pipetted up and down. HRP-Streptavidin concentrate was diluted 4,000 – fold with 1x Assay Diluent B.

Figure 2: Dilution of adiponectin standard



ASSAY PROCEDURE:

1. All reagents and samples were allowed to attain the room temperature before use.

2. 100 µl of each standard and sample were added into appropriate wells and incubated for 2.5 hours at room temperature.

3. The solution was discarded and washed 4 times with 1 x Wash Solution. Washed by filling each well with Wash Buffer (300 µl) using a multi-channel Pipette. After the last wash, any remaining Wash Buffer was removed by aspirating or decanting. The plate was inverted and blotted against clean paper towels.

4. Then 100 µl of 1 x prepared biotinylated antibody were added to each well. Incubated for 1 hour at room temperature with gentle shaking.

5. The solution was discarded. The wash step was repeated as in step 3.

6. 100 µl of prepared Streptavidin solution were added to each well. Incubated for 45 minutes at room temperature with gentle shaking.

7. The solution was discarded. Repeated the wash as in step 3.

8. Then 100 µl of TMB One-step Color Substrate Reagent (Item H) were added to each well. Incubated for 30 minutes at room temperature.

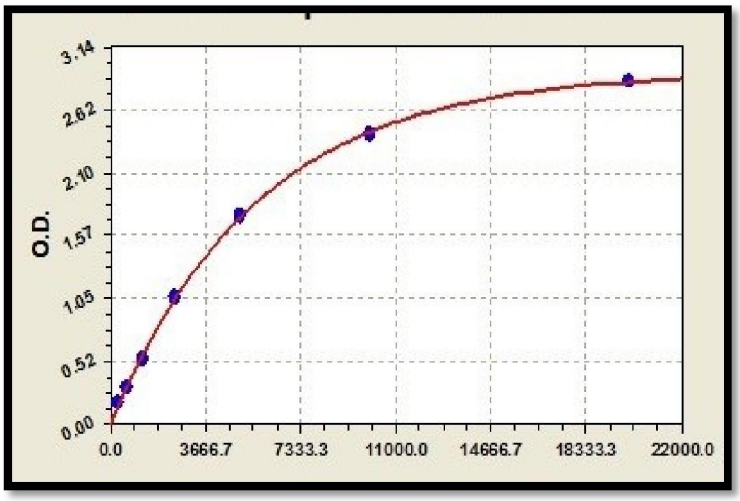
9. 50 µl of Stop Solution (Item I) were added to each well. The color in the wells changed from blue to yellow.

10. The optical density of each well was determined immediately using a micro plate reader set to 450 nm.

Calculation of results:

The optical density of each sample was plotted against its concentration and a curve was drawn through the points. Because the samples were diluted, the concentrations was read from the standard curve and multiplied by the dilution factor.

Figure 3: Adiponectin standard curve



ASSAY PROCEDURE SUMMARY:

Reagents, samples and standards were prepared as instructed

↓

100 µl sample was added to each well.

Incubated for 2.5 hours at room temperature

↓

Aspirated and washed four times

↓

100 µl prepared biotin antibody to each well.

Incubated for 1 hour at room temperature.

↓

Aspirated and washed four times

↓

100 µl prepared Streptavidin solution.

Incubated 45 minutes at room temperature.

↓

100 µl TMB One-step Substrate Reagent to each well,

Incubated 30 minutes at room temperature.

↓

50 µl Stop Solution to each well

↓

Absorbance read at 450 nm immediately.

Photograph 1 : Healthy periodontium - Control group

Photograph 2 : T2DM with Generalised chronic periodontitis - Study group-I

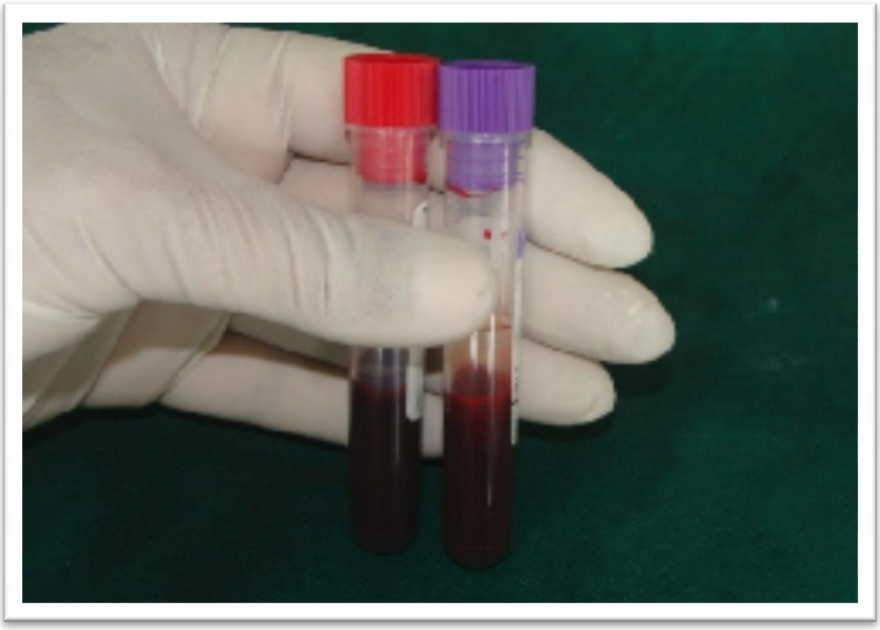
Photograph 3 : Measurement of probing depth with William's periodontal probe

Photograph 4: Orthopantomogram

Photograph 5: After Non-surgical periodontal therapy – Study group-II

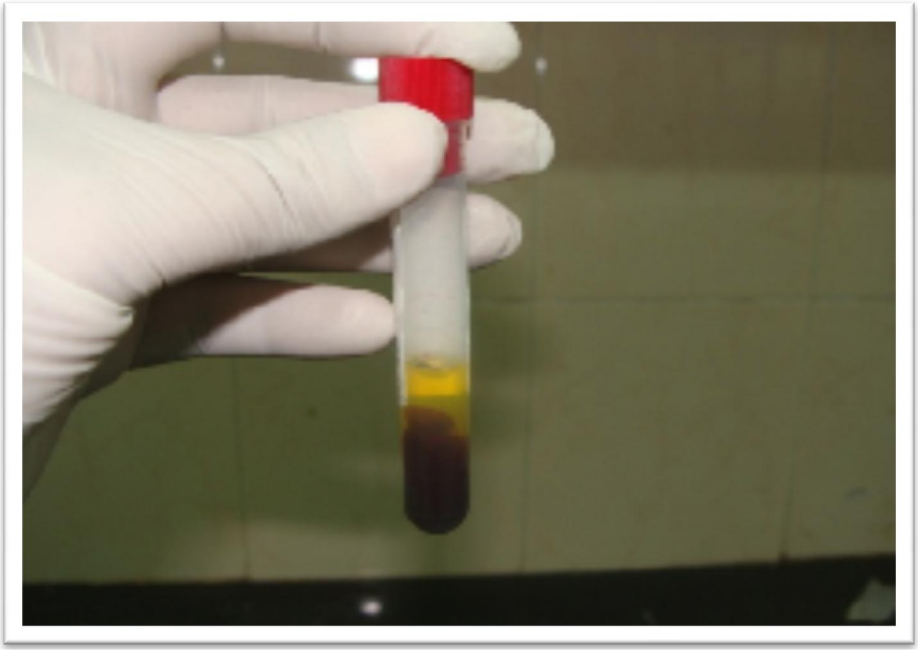
Photograph 6: Armamentarium for periodontal examination

Photograph 7: Collection of blood sample

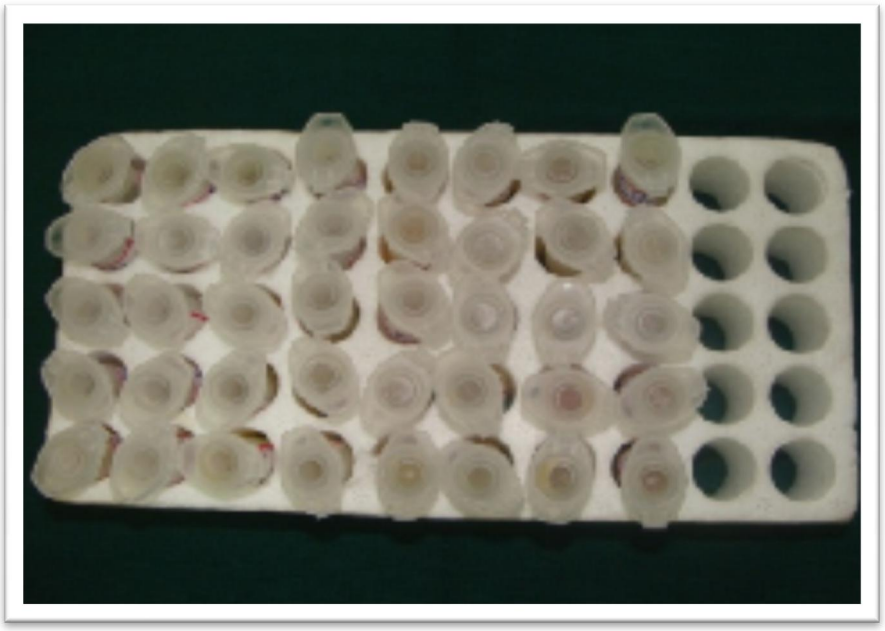


Photograph 8: Collected blood sample

Photograph 9: Centrifuge machine



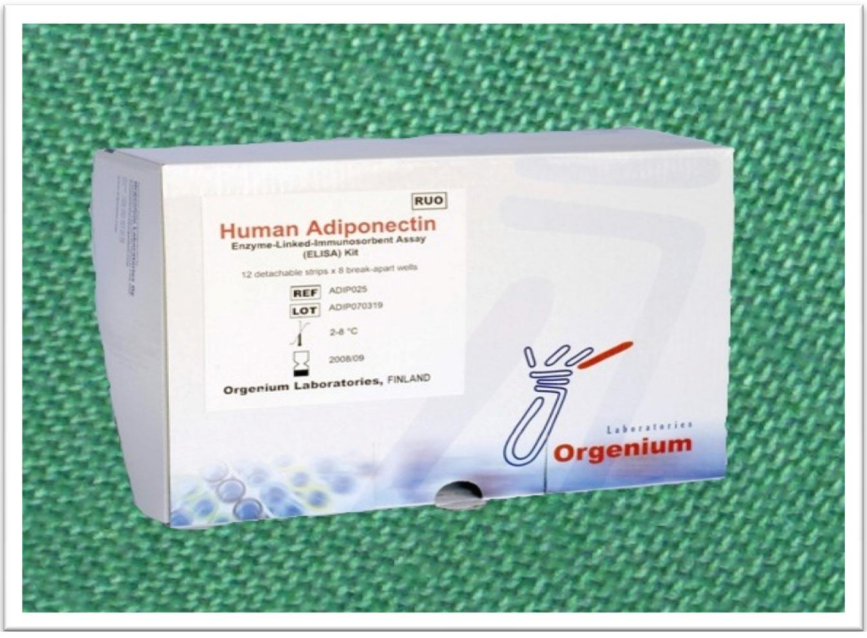
Photograph 10: Centrifuged serum



Photograph 11: Serum in Eppendorf microfuge tube

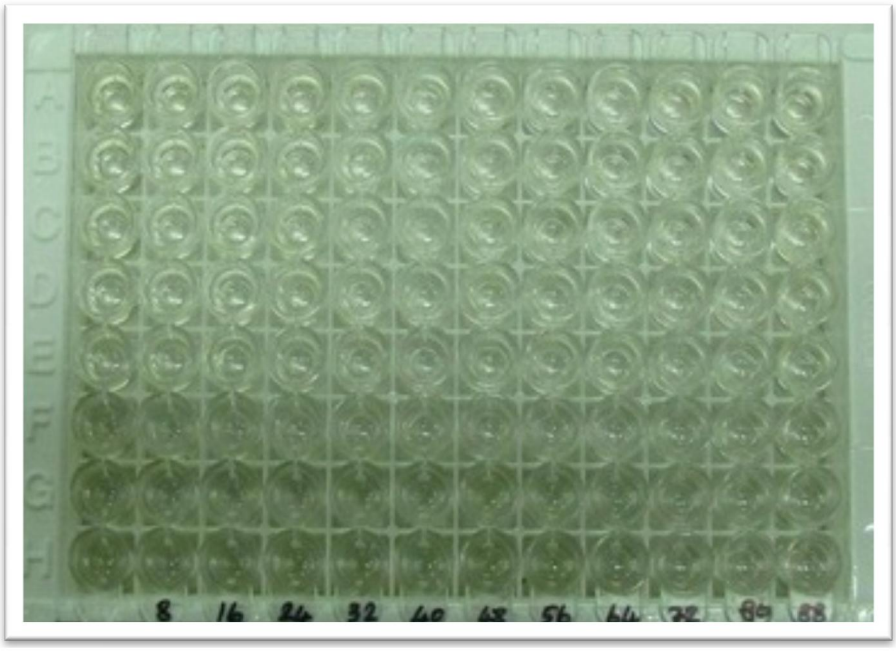
Photograph 12: Armamentarium for sample transportation

Photograph 13 - C



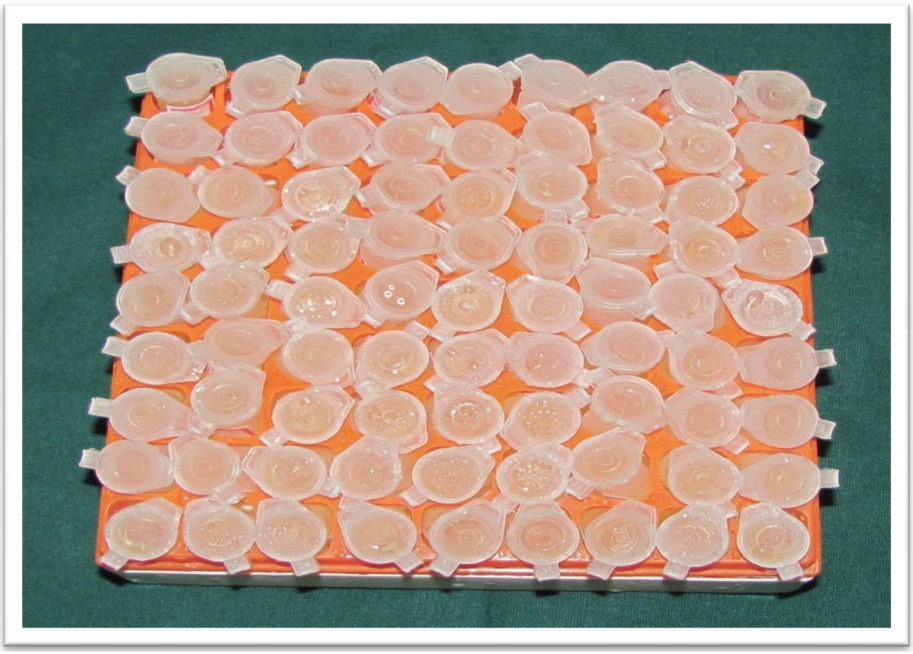
Photograph 14: ELISA kit

Photograph 15: ELISA kit contents



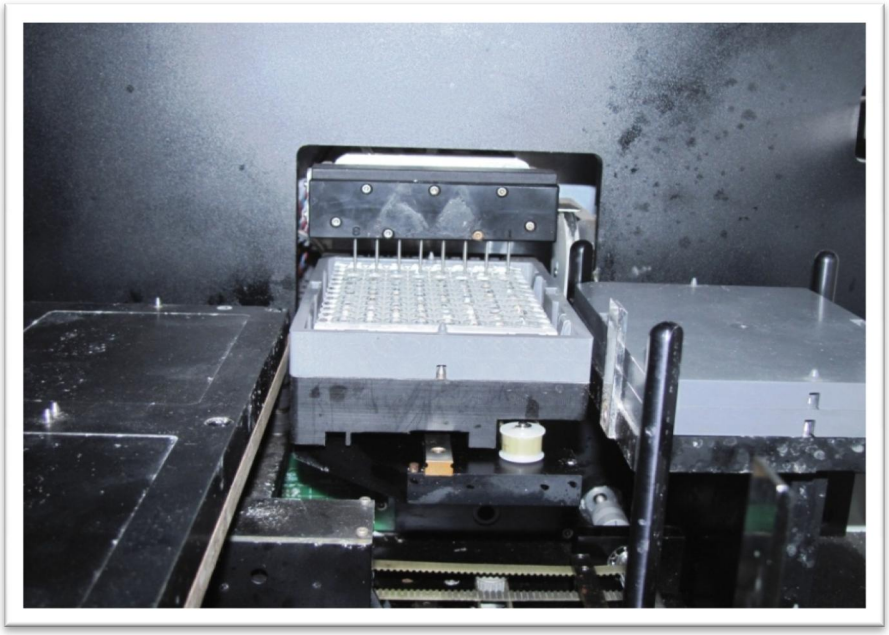
Photograph 16: 96 well microplate of ELISA kit

Photograph 17: Micropipette

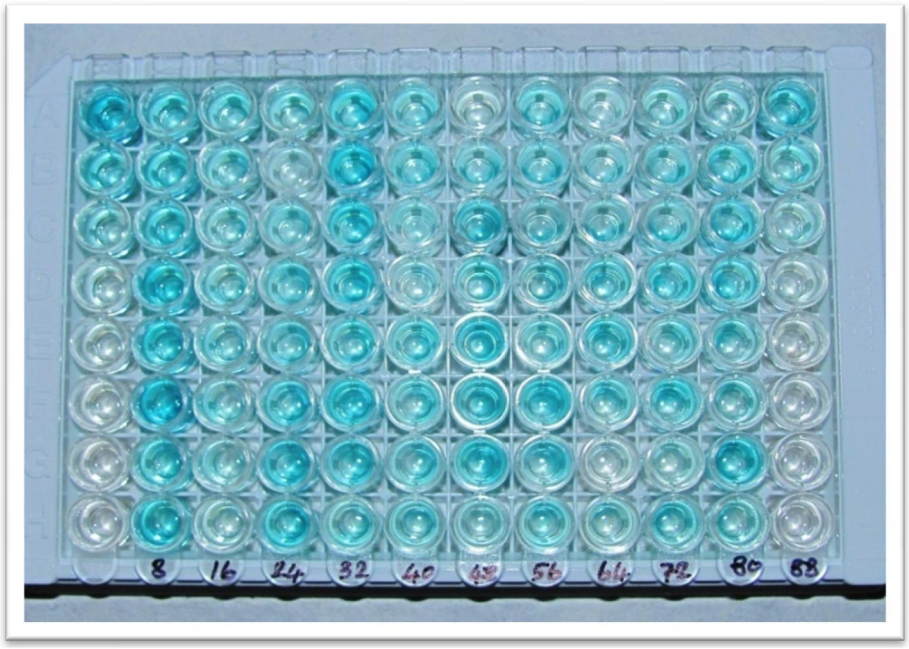


Photograph 18: Collected serum samples for assay

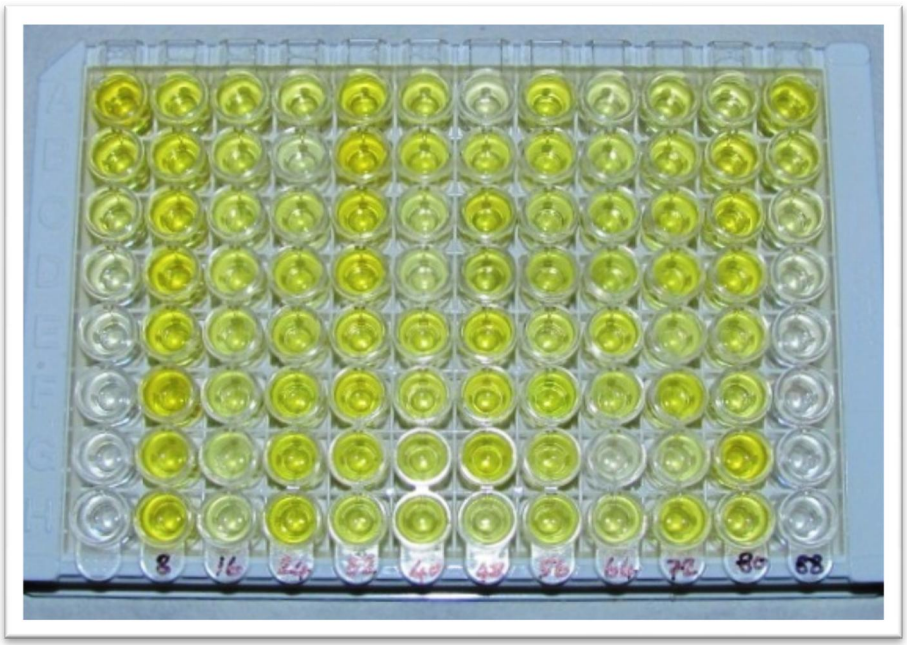
Photograph 19: ELISA reader and washer



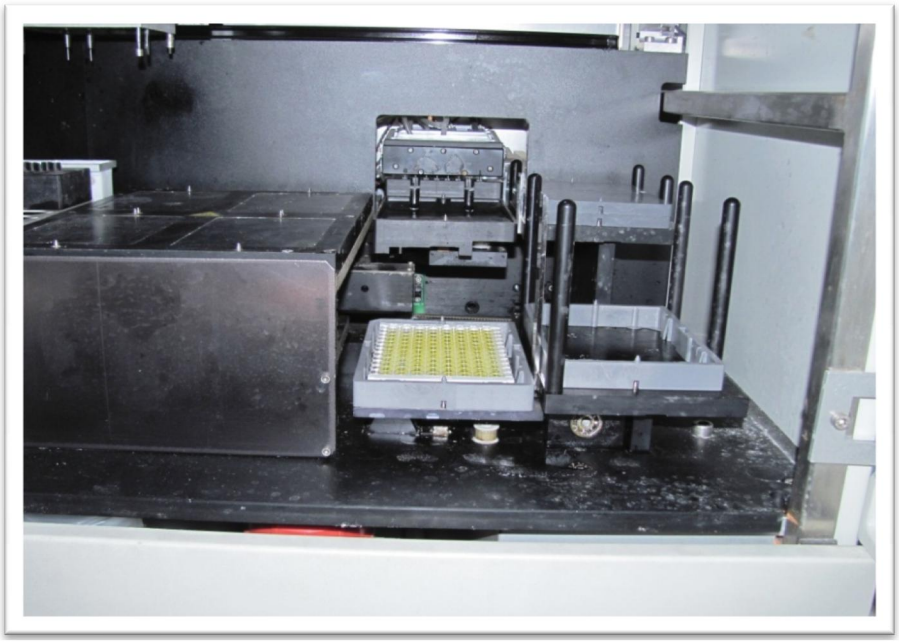
Photograph 20: 96 well microplate on autowasher



Photograph 21: 96 well microplate with conjugate solution



Photograph 22: 96 well microplate after adding substrate solution



Photograph 23: 96 well microplate on ELISA reader

Photograph 24: Estimation of fasting blood glucose level by Glucometer

STATISTICAL ANALYSIS

The statistical analysis was done using the computer software program SPSS (Statistical Package for Social Sciences) for Windows version 17.

Mean and Standard Deviation were estimated for different variables in each group.

Mean values were compared between levels before & after treatment using ***Student's Paired t-test.***

Pearson's chi-square test was done to compare the gender distribution between the two groups.

Kendall's tau-b rank correlation coefficient test was used to analyze the correlation between the clinical parameters, fasting blood sugar level and adiponectin level.

In the present study, ***P-value <0.05*** was considered as the level of significance**.**

STATISTICAL FORMULAE USED FOR DATA ANALYSIS

Student's Paired t-test:

The Paired t-test was used to compare the statistical significance of a possible difference between the means of same group on some dependent variable and the two groups were dependent of one another.

The formula for the Paired t-test was

$$t = \frac{\Sigma d}{\sqrt{\frac{n\left(\Sigma d^2\right) - \left(\Sigma d\right)^2}{n-1}}}$$

The top of the formula is the sum of the differences (i.e. the sum of d). The bottom of the formula reads as:

The square root of the following: n times the sum of the differences squared minus the sum of the squared differences, all over n-1.

The sum of the squared differences: ∑d2 means take each difference in turn, square it, and add up all those squared numbers.

The sum of the differences squared: (∑d)2 means add up all the differences and square the result.

The **P value** or calculated probability was the estimated probability of rejecting the null hypothesis (H0) of a study question when that hypothesis was true. The smaller the p-value, the more significant the result was said to be. All *P*-values are two tailed, and confidence intervals were calculated at the 95% level. Differences between the two populations were considered significant when $p < 0.05$.

Pearson's Chi-square Test:

The formula used was

$$\chi^2 = \sum_{i=1}^{n} \left\{ \frac{(O-E)^2}{E} \right\}$$

Where O = Observed frequency in a cell

E = Expected frequency in a cell

Kendall's tau-b Test:

Kendall's tau-b was used to measure the strength of relationship between the two variables and the coefficient was used to test for statistical dependence. Values of tau-b ranges from −1 (100% negative association or perfect inversion) to +1 (100% positive association or perfect agreement). A value of zero indicates the absence of association.

The Kendall tau-b coefficient is defined as:

$$\tau_B = \frac{n_c - n_d}{\sqrt{(n_0 - n_1)(n_0 - n_2)}}$$

Where,

$$\begin{aligned} n_0 &= n(n-1)/2 \\ n_1 &= \textstyle\sum_i t_i(t_i-1)/2 \\ n_2 &= \textstyle\sum_j u_j(u_j-1)/2 \\ t_i &= \text{Number of tied values in the } i^{th} \text{ group of ties for the first quantity} \\ u_j &= \text{Number of tied values in the } j^{th} \text{ group of ties for the second quantity} \end{aligned}$$

RESULTS

Hundred subjects were included in this present study. The subjects were categorized into three groups as follows:

50 subjects with healthy periodontium as the Control group

50 subjects type-2 diabetes mellitus with moderate to severe generalized chronic periodontitis as the Study group and it is considered as the following:

Baseline level (Pre-treatment) – Study Group-I

After initial treatment (Post-treatment) – Study Group-II

Table 1, 2 and 3 shows the master chart of the control group, study group- I and II with the clinical parameters, fasting blood sugar level and serum adiponectin level.

Table 4 and Figure 4 shows the comparison of age between the study and control group. The mean age in the study group was 46 years and 41 years in the control group respectively. There was no statistical significant difference in the distribution of sex in both the groups.

Table 5 and Figure 5 shows the comparison of gender distribution between the study and control group. The males constituted 46% while females constituted 54% in the study group. Both males and females constituted about 44% and 56% respectively in the control group. There was no statistical significant difference in distribution of the gender between the groups.

Table 6 and Figure 6 shows the comparison of clinical parameter mean Plaque Index between the study group I and II. The mean Plaque Index score in the study group-I was 2.494±0.147 and 1.187 ±0.079 in the study group-II, which was statistically highly significant (p=0.000).

Table 6 and Figure 7 shows the comparison of clinical parameter mean % of sites with Bleeding on Probing between the study group I and II. The mean % of sites with Bleeding on Probing was 90.407±5.385 in the study group-I and 8.563±3.289 in the study group-II, which was statistically highly significant (p=0.000).

Table 7 and Figure 8 shows the comparison of mean adiponectin levels between the study group I and II. The mean adiponectin levels in serum were 7.52±2.96 µg/mL in the study group-I and 8.39±2.52 µg/mL in the study group-II, which was statistically highly significant difference in serum adiponectin levels between the study group I and II. (p=0.000)

Table 8 and Figure 9 shows the comparison of mean CAL between study group-I and study group-II. The mean CAL was 7.54±1.804mm in study group-I and 2.74±0.655mm in study group-II. There was statistically highly significant difference in CAL between study group-I and II. (p=0.000)

Table 9 and Figure 10 shows the comparison of mean PPD between study group-I and study group-II. The mean PPD was 7.13±1.738mm in study group-I and 2.283±0.423mm in study group-II, which was statistically highly significant difference in PPD between study group-I and II. (p=0.000)

Table 10 and Figure 11 shows the comparison of mean FBS level between study group-I and study group-II. The mean FBS level was 183.20±24.982mg/mL in study group-I and 175.20±20.360mg/mL in study group-II. There was statistically highly significant difference in FBS level between study group-I and II. (p=0.000)

Table 11 shows the correlation between the clinical parameters, fasting blood sugar level and adiponectin level in both study group I and II. A linear negative correlation was found between PPD and adiponectin level in the study group I and II, which was statistically highly significant (p=0.000) **(Figure 12, 13)**. A linear negative correlation was found between CAL and adiponectin level in the study group I and II, which was statistically highly significant (p=0.000) **(Figure 14, 15)**. A linear negative correlation was found between fasting blood sugar level and adiponectin level in the study group I and II, which was statistically highly significant correlation (p=0.000) **(Figure 16, 17)**.

Table 1: MASTER CHART - CONTROL GROUP

CASE NO	AGE	SEX	PI	% OF SITES BOP	PPD (mm)	CAL (mm)	Adiponectin (µg/mL)	Fasting blood sugar (mg/ml)
C1	43	M	0.6	5.50	1.9	0	15.1	94
C2	38	F	0.7	6.60	1.68	0	16.2	90
C3	41	F	0.7	7.80	1.93	0	15.8	99
C4	38	F	0.5	4.28	1.30	0	17.1	81
C5	51	M	0.4	3.99	1.65	0	18.2	94
C6	45	F	0.5	3.88	1.90	0	19.9	89
C7	50	M	0.6	10.42	2.27	0	9.1	107
C8	45	F	0.6	4.46	1.60	0	16.9	89
C9	50	M	0.6	4.00	2.18	0	9.5	108
C10	48	F	0.7	4.77	2.28	0	11.9	101
C11	52	M	0.5	6.82	1.9	0	13.4	99
C12	48	F	0.6	6.00	1.7	0	14.5	98
C13	40	F	0.7	4.22	1.6	0	16.7	90
C14	54	F	0.5	6.48	2.5	0	62	109
C15	48	F	0.6	5.45	1.6	0	16.6	91
C16	40	M	0.5	7.93	1.5	0	14.8	93
C17	46	F	0.6	3.60	1.5	0	18.1	87
C18	48	M	0.7	6.20	1.6	0	17.0	91
C19	47	F	0.6	5.50	1.5	0	18.9	88
C20	36	F	0.7	4.60	1.4	0	17.1	82
C21	38	M	0.5	5.17	1.7	0	15.1	86
C22	50	F	0.7	4.00	1.6	0	21.4	84
C23	45	M	0.6	5.50	1.8	0	14.4	90
C24	45	F	0.5	6.88	1.9	0	14.9	97
C25	51	M	0.6	4.70	1.5	0	19.4	82
C26	39	F	0.5	4.00	1.7	0	16.9	88
C27	56	F	0.6	7.55	2.0	0	13.9	99
C28	55	M	0.6	7.94	2.19	0	9.3	106
C29	37	F	0.5	4.00	1.9	0	17.0	84
C30	48	M	0.5	7.66	2.3	0	7.0	109
C31	38	M	0.6	4.09	1.6	0	15.9	82
C32	36	M	0.5	8.71	1.9	0	15.3	88
C33	41	M	0.6	4.00	1.5	0	15.8	81
C34	49	F	0.7	5.50	1.9	0	18.1	86
C35	38	F	0.7	6.50	1.6	0	17.2	83
C36	36	F	0.6	9.64	1.9	0	15.9	88
C37	50	F	0.6	5.40	2.3	0	8.0	108
C38	51	M	0.5	8.58	2.3	0	7.9	105

C39	38	F	0.5	4.60	2.25	0	7.9	106
C40	46	M	0.7	5.10	1.7	0	12.9	93
C41	50	F	0.6	6.50	1.6	0	21.0	87
C42	39	M	0.6	5.80	1.2	0	15.1	87
C43	51	F	0.6	6.10	2.4	0	6.5	109
C44	39	F	0.7	7.70	2.3	0	7.9	107
C45	54	F	0.7	7.60	2.2	0	8.9	103
C46	49	M	0.6	6.40	1.9	0	12.6	99
C47	39	M	0.7	7.60	2.25	0	6.3	107
C48	52	M	0.6	8.90	2.0	0	8.1	103
C49	48	F	0.6	9.10	2.3	0	8.3	108
C50	40	M	0.6	5.00	1.7	0	13.6	94

Table 1 : MASTER CHART - STUDY GROUP – I

CASE NO	AGE	SEX	PI	% OF SITES BOP	PPD (mm)	CAL (mm)	Adiponectin (µg/mL)	FBS (mg/ml)
SP1	40	F	2.33	78.90	4.10	4.15	13.2	142
SP2	38	F	2.24	89.06	5.18	5.44	8.9	178
SP3	43	F	2.07	91.45	6.89	7.94	8.0	185
SP4	46	F	2.21	85.15	9.21	9.90	3.9	223
SP5	44	M	2.2	90.62	7.57	8.46	5.1	199
SP6	45	M	2.3	55.91	6.06	6.17	9.8	162
SP7	40	M	2.46	87.54	6.22	7.98	9.1	170
SP8	47	M	2.5	91.40	9.39	9.99	3.7	221
SP09	43	M	2.42	65.33	8.16	8.90	5.2	190
SP10	45	F	2.44	81.67	6.00	7.99	9.6	169
SP11	38	F	2.48	92.97	4.10	4.05	11.2	157
SP12	46	M	2.77	89.71	8.90	9.10	5.1	197
SP13	40	M	2.57	57.64	4.30	4.50	11.9	140
SP14	45	M	2.37	91.40	6.08	7.90	7.0	181
SP15	41	F	2.56	64.86	5.19	5.62	11.3	143
SP16	44	F	2.63	92.19	9.31	9.50	4.2	218
SP17	39	M	2.36	85.16	5.27	5.53	10.4	157
SP18	41	F	2.57	91.66	5.97	6.78	9.1	174
SP19	45	F	2.54	93.55	5.09	5.58	10.2	155
SP20	49	M	2.1	100.00	9.34	9.69	3.6	220
SP21	40	F	2.5	95.00	5.41	5.83	14.9	151
SP22	43	F	2.41	75.08	7.29	7.92	9.0	172
SP23	38	M	2.8	94.50	5.80	6.38	9.4	168
SP24	47	M	2.5	96.09	7.98	8.52	8.0	173
SP25	39	F	2.74	77.32	6.05	6.73	10.1	168
SP26	48	F	2.52	92.18	8.97	9.29	4.3	209
SP27	39	F	2.68	49.78	4.10	4.25	13.0	132
SP28	49	F	2.5	88.06	8.93	9.52	3.9	214
SP29	41	F	2.35	92.06	5.51	6.01	9.0	168
SP30	38	M	2.52	89.06	4.32	4.50	10.4	153

SP31	50	M	2.44	67.16	8.81	9.81	3.8	215
SP32	44	F	2.6	99.20	9.05	9.30	4.3	208
SP33	41	F	2.11	96.02	8.45	8.93	5.1	195
SP34	44	F	2.6	88.44	8.79	8.53	6.4	184
SP35	42	M	2.5	98.17	6.13	6.33	6.9	181
SP36	51	F	2.6	99.13	8.15	8.85	7.8	179
SP37	47	M	2.1	91.70	8.97	9.41	5.1	192
SP38	48	F	2.5	98.17	8.90	9.23	4.5	215
SP39	42	M	2.23	93.21	6.95	7.10	7.4	184
SP40	42	F	2.6	92.03	7.85	6.80	6.6	189
SP41	41	F	2.5	95.00	6.53	5.53	9.1	181
SP42	46	F	2.41	75.08	8.45	7.45	6.7	193
SP43	49	M	2.8	94.50	8.45	9.29	4.4	204
SP44	46	F	2.5	96.09	9.14	9.25	4.3	218
SP45	47	M	2.74	77.32	9.51	9.98	3.5	227
SP46	42	M	2.52	92.18	6.43	6.78	8.3	174
SP47	38	M	2.68	49.78	5.58	5.83	10.5	155
SP48	44	F	2.5	88.06	8.89	9.17	4.2	220
SP49	40	M	2.35	92.06	5.78	6.37	9.3	167
SP50	46	M	2.52	89.06	8.85	8.93	5.3	190

Table 3 : MASTER CHART - STUDY GROUP -II

CASE NO	AGE	SEX	PI	% OF SITES BOP	PPD (mm)	CAL (mm)	Adiponectin (µg/mL)	FBS (mg/mL)
PO1	40	F	0.8	7.50	1.55	1.65	13.4	145
PO2	38	F	0.6	5.60	1.95	2.05	9.4	172
PO3	43	F	0.7	6.80	2.69	2.98	8.9	177
PO4	46	F	1.15	9.28	2.21	3.82	5.9	206
PO5	44	M	0.8	5.99	2.17	2.46	6.8	189
PO6	45	M	0.5	3.88	2.06	2.17	10.6	156
PO7	40	M	0.85	7.45	2.18	2.28	9.2	168
PO8	47	M	1.3	9.86	2.39	3.42	5.6	207
PO9	43	M	0.6	6.50	2.16	2.20	6.4	178
PO10	45	F	0.75	5.75	1.90	2.0	10.5	161
PO11	38	F	0.5	4.82	1.65	1.80	11.8	151
PO12	46	M	0.9	8.10	1.95	2.55	6.5	183
PO13	40	M	0.6	6.22	2.15	2.90	12.2	148
PO14	45	M	0.8	8.48	2.00	2.85	8.0	173
PO15	41	F	0.6	5.45	1.95	2.05	11.7	139
PO16	44	F	0.98	11.93	2.01	3.55	4.7	211
PO17	39	M	0.5	3.60	1.72	1.90	10.8	152
PO18	41	F	0.7	8.20	1.85	2.50	9.6	169
PO19	45	F	0.8	5.50	2.09	2.18	10.6	151
PO20	49	M	1.35	11.95	2.74	3.90	6.3	201

PO21	40	F	0.5	5.17	1.61	1.83	15.4	148
PO22	43	F	0.7	6.00	2.29	2.92	9.7	165
PO23	38	M	0.45	5.50	2.30	2.32	9.9	163
PO24	47	M	0.45	5.88	2.98	2.52	8.8	165
PO25	39	F	0.65	11.10	2.05	2.73	10.4	166
PO26	48	F	0.9	7.55	2.05	3.25	5.8	195
PO27	39	F	0.6	6.65	1.50	1.80	13.1	131
PO28	49	F	1.45	9.94	2.93	3.72	5.6	200
PO29	41	F	0.5	4.00	1.90	2.25	9.7	162
PO30	38	M	0.5	7.66	2.82	2.97	10.5	150
PO31	50	M	0.55	9.95	2.81	3.91	5.6	201
PO32	44	F	0.8	8.71	2.25	3.05	6.0	198
PO33	41	F	0.6	4.00	2.45	2.93	6.1	186
PO34	44	F	0.9	7.50	2.79	2.53	7.2	175
PO35	42	M	0.7	6.50	2.93	2.53	7.5	176
PO36	51	F	1.6	15.95	2.95	2.20	8.5	172
PO37	47	M	0.6	9.40	2.29	3.10	5.8	183
PO38	48	F	0.5	8.58	2.90	3.83	5.6	202
PO39	42	M	0.74	5.00	2.95	2.0	8.4	177
PO40	42	F	0.75	6.50	2.15	3.17	7.0	183
PO41	41	F	0.58	7.80	2.22	2.52	9.1	178
PO42	46	F	0.75	8.45	2.47	3.50	7.1	181
PO43	49	M	1.35	9.65	2.05	3.25	5.8	194
PO44	46	F	0.45	10.10	2.14	3.75	5.5	203
PO45	47	M	0.7	11.45	2.51	3.98	5.7	204
PO46	42	M	0.85	6.50	2.43	2.78	8.8	167
PO47	38	M	0.92	5.45	1.90	2.20	10.7	151
PO48	44	F	0.7	9.75	2.89	2.17	5.5	204
PO49	40	M	0.5	6.00	1.95	2.70	9.9	162
PO50	46	M	0.6	6.45	2.85	3.23	5.9	181

Table 4: Comparison of age between study and control group

	Group	N	Mean	Std. Deviation	P-value
Age (in years)	**Study**	50	44.93	2.586	**.073 (NS)**
	Control	50	42.65	5.385	

NS - NOT SIGNIFICANT; P >0.05

Table 5: Comparison of gender between study and control group

			Group		Total	P-value
			Study	**Control**		
Sex	**Male**	**Count**	23	22	45	**.501 (NS)**
		% within Sex	51.1%	48.9%	100.0%	
		% within Group	46.0%	44.0%	45.0%	
	Female	**Count**	27	28	55	
		% within Sex	49.1%	50.9%	100.0%	
		% within Group	54.0%	56.0%	55.0%	
Total		**Count**	50	50	100	
		% within Sex	50.0%	50.0%	100.0%	
		% within Group	100.0%	100.0%	100.0%	

Table 6: Comparison of clinical parameters between study group I and II

	Study group – I		Study group - II		P-value
	Mean	SD	Mean	SD	
Plaque Index	2.494	0.147	0.987	0.079	**.000 (S)**
% of sites with bleeding on probing	90.407	5.385	7.563	2.289	**.000 (S)**

P < 0.001; S-SIGNIFICANT

Table 7: Comparison of mean Adiponectin level between study group-I and study group-II

	Mean	N	Std. Deviation	Std. Error Mean
Pair 1 CONTROL	15.0260	50	7.92613	1.12092
PREOP	7.5200	50	2.96090	.41873
Pair 2 CONTROL	15.0260	50	7.92613	1.12092
POSTOP	8.3900	50	2.52411	.35696
Pair 3 PREOP	7.5200	50	2.96090	.41873
POSTOP	8.3900	50	2.52411	.35696

		df	Sig. (2-tailed)
Pair 1	CONTROL - PREOP	49	.000
Pair 2	CONTROL - POSTOP	49	.000
Pair 3	PREOP - POSTOP	49	.000

P <0.001; Sig – SIGNIFICANT (2-tailed)

Table 8: Comparison of mean CAL between study group-I and study group-II

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	PREOP	7.5398	50	1.80421	.25515
	POSTOP	2.7370	50	.65517	.09266

		df	Sig. (2-tailed)
Pair 1	PREOP - POSTOP	49	.000

P <0.001; Sig – SIGNIFICANT (2-tailed)

Table 9: Comparison of mean PPD between study group-I and study group-II

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	CONTROL	1.8576	50	.32137	.04545
	PREOP	7.1270	50	1.73897	.24593
Pair 2	CONTROL	1.8576	50	.32137	.04545
	POSTOP	2.2836	50	.42347	.05989
Pair 3	PREOP	7.1270	50	1.73897	.24593
	POSTOP	2.2836	50	.42347	.05989

		df	Sig. (2-tailed)
Pair 1	CONTROL - PREOP	49	.000
Pair 2	CONTROL - POSTOP	49	.000
Pair 3	PREOP - POSTOP	49	.000

P <0.001; Sig – SIGNIFICANT (2-tailed)

Table 10: Comparison of mean FBS level between study group-I and study group-II

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	CONTROL	94.5800	50	9.17648	1.29775
	PREOP	183.2000	50	24.98244	3.53305
Pair 2	CONTROL	94.5800	50	9.17648	1.29775
	POSTOP	175.2000	50	20.36002	2.87934
Pair 3	PREOP	183.2000	50	24.98244	3.53305
	POSTOP	175.2000	50	20.36002	2.87934

		df	Sig. (2-tailed)
Pair 1	CONTROL - PREOP	49	.000
Pair 2	CONTROL - POSTOP	49	.000
Pair 3	PREOP - POSTOP	49	.000

Table 11: Correlation between clinical parameters, fasting blood sugar and adiponectin level in study group I and II

		Adiponectin (µg/mL)	
		Study group -I	**Study group – II**
PPD (mm)	**Correlation Coefficient**	-.795	-.390
	Sig. (2 tailed)	**.000 (S)**	**.000 (S)**
	N	50	50
CAL (mm)	**Correlation Coefficient**	-.778	-.581
	Sig. (2 tailed)	**.000 (S)**	**.000 (S)**
	N	50	50
FBS (mg/ml)	**Correlation Coefficient**	-.893	-.848
	Sig. (2 tailed)	**.000 (S)**	**.000 (S)**
	N	50	50

P <0.001; S – SIGNIFICANT (2-tailed)

Figure 4: Comparison of age between Study and Control group

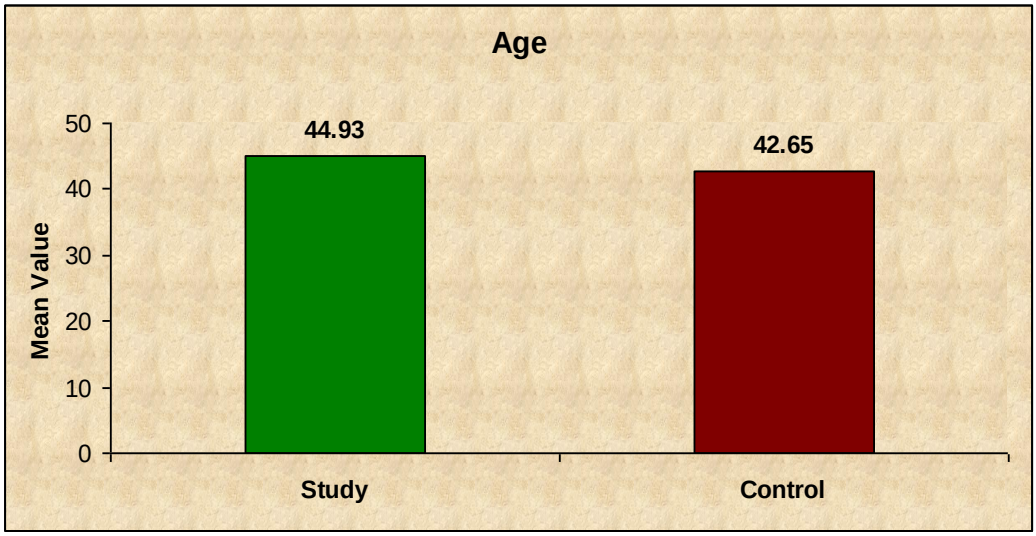


Figure 5: Comparison of gender between Study and Control group

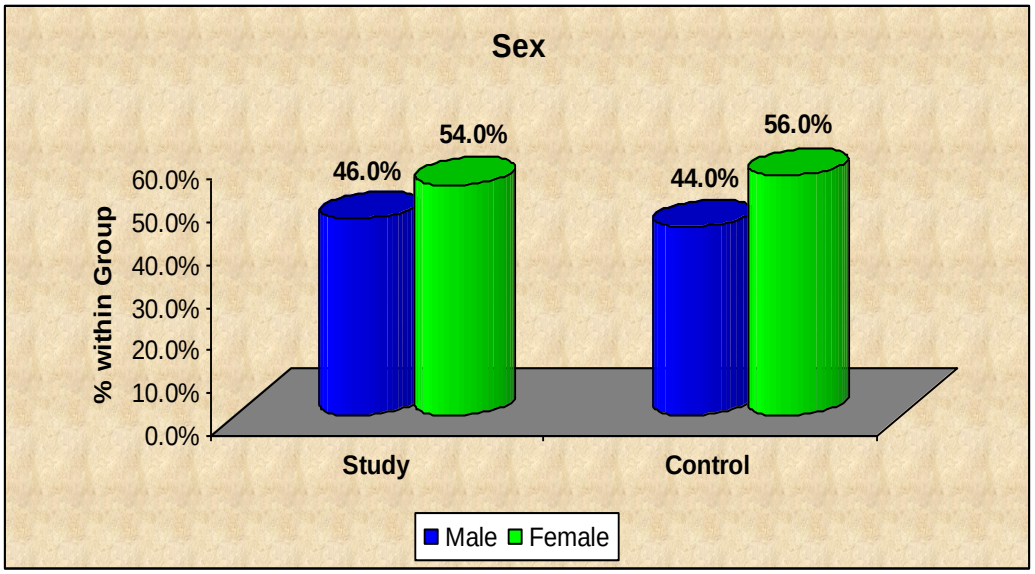


Figure 6: Comparison of mean Plaque Index between Study group I and II

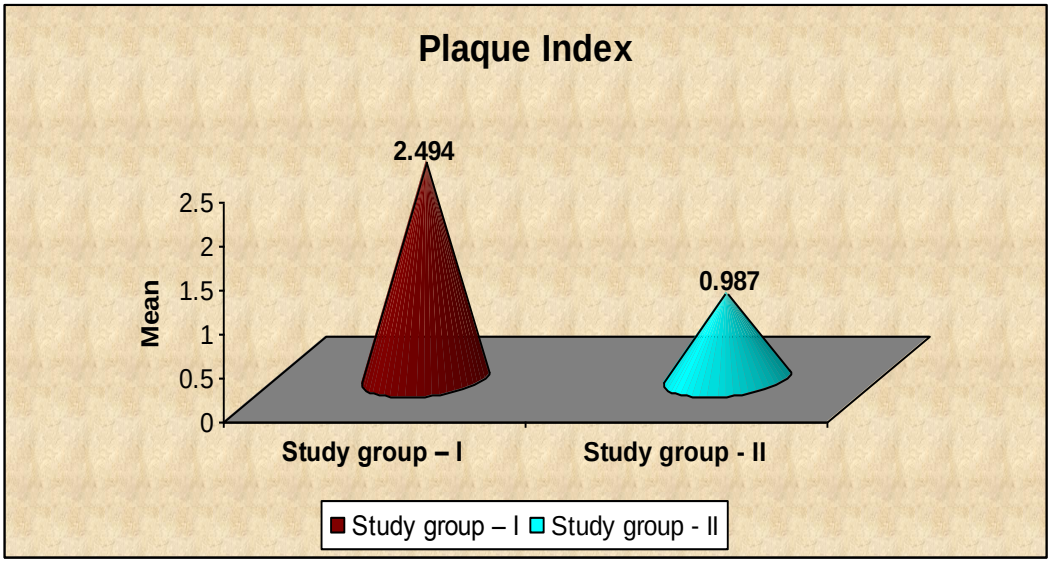


Figure 7: Comparison of mean % of sites with BOP between Study group I and II

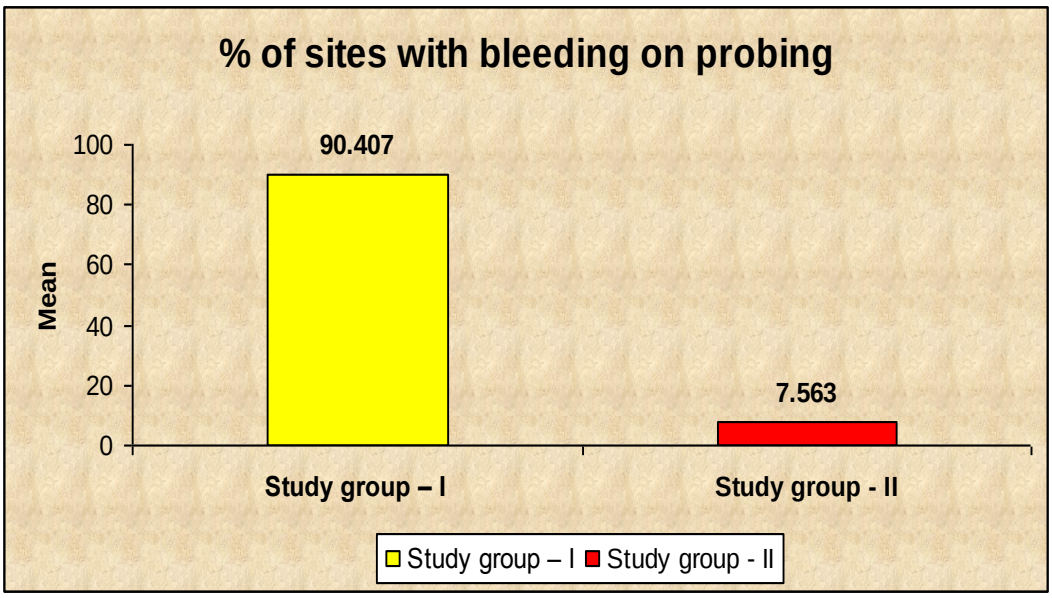


Figure 8: Comparison of mean adiponectin level in Study group I and II

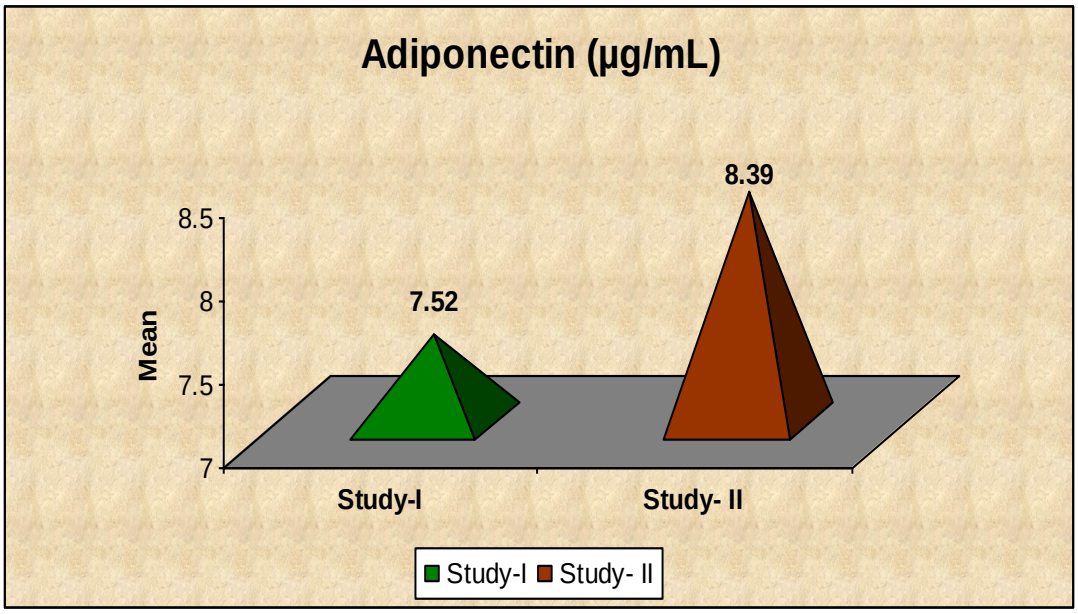


Figure 9: Comparison of mean CAL between Study group I and II

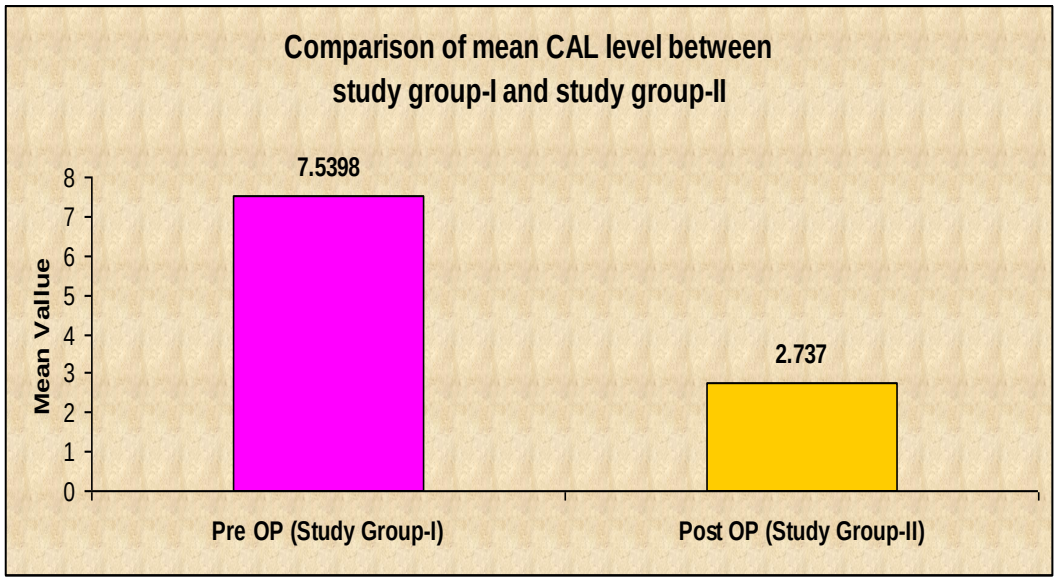


Figure 10: Comparison of mean PPD between Study group I and II

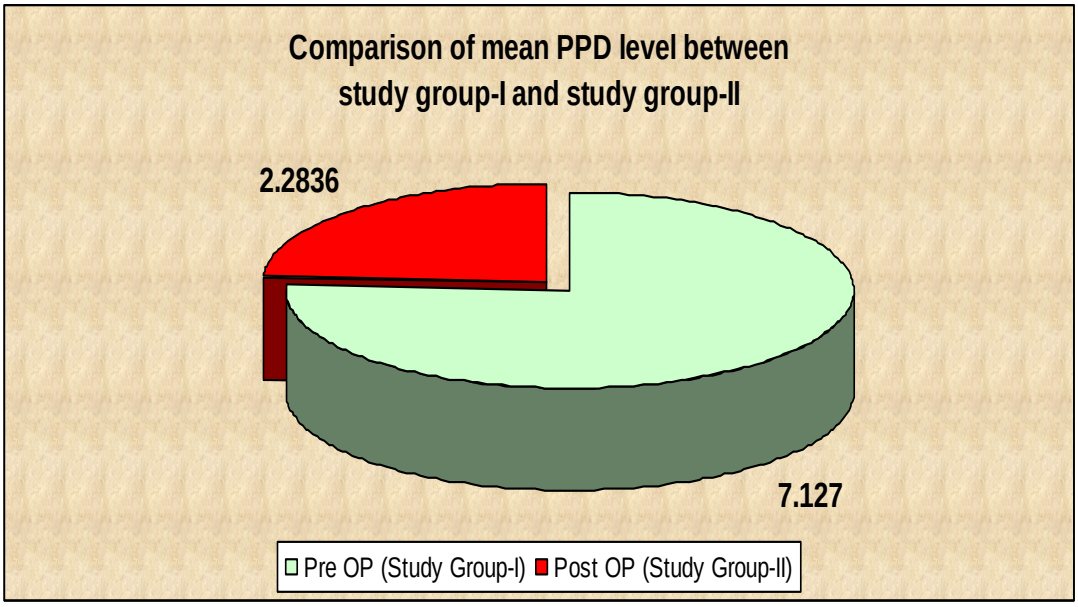


Figure 11: Comparison of mean FBS level between Study group I and II

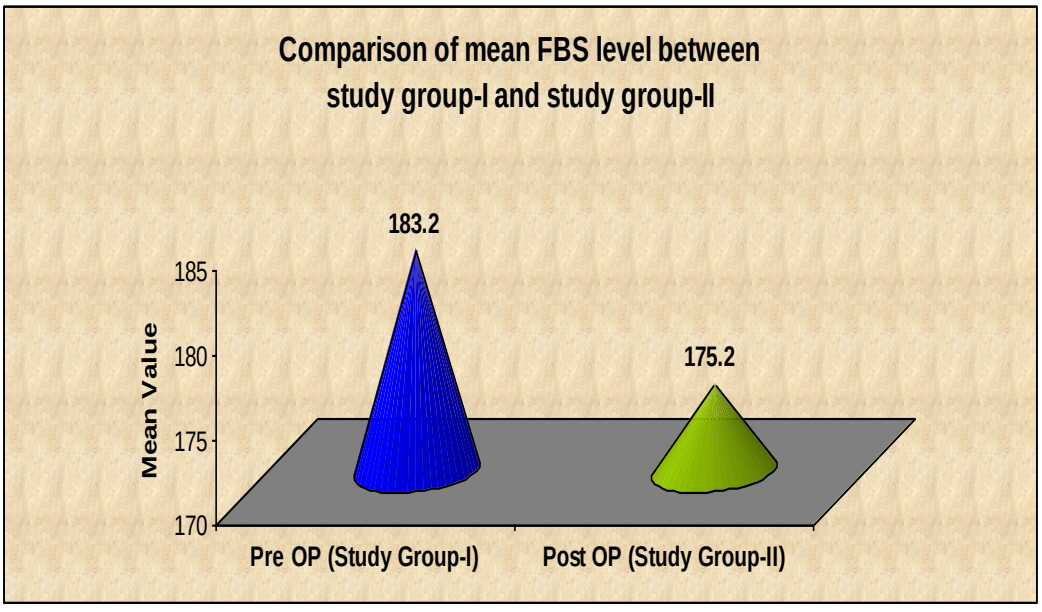


Figure 12: Correlation of PPD and Adiponectin level in Study group-I

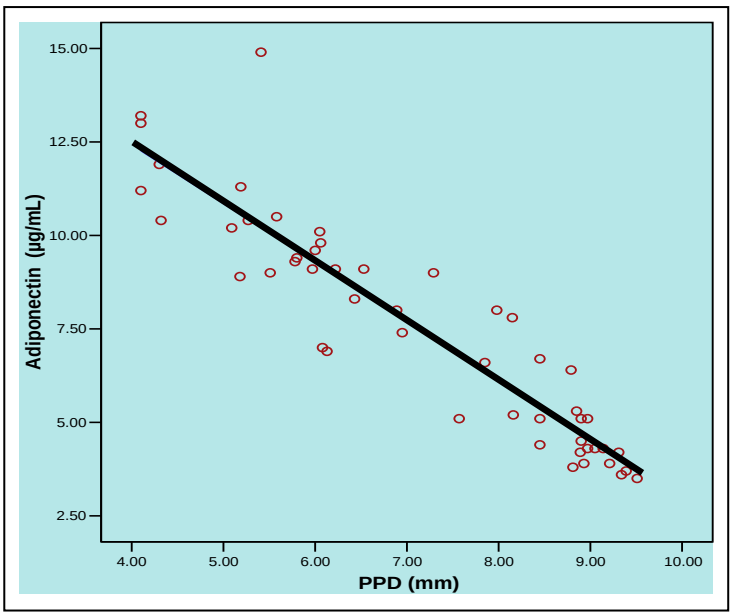


Figure 13: Correlation of PPD and Adiponectin level in Study group-II

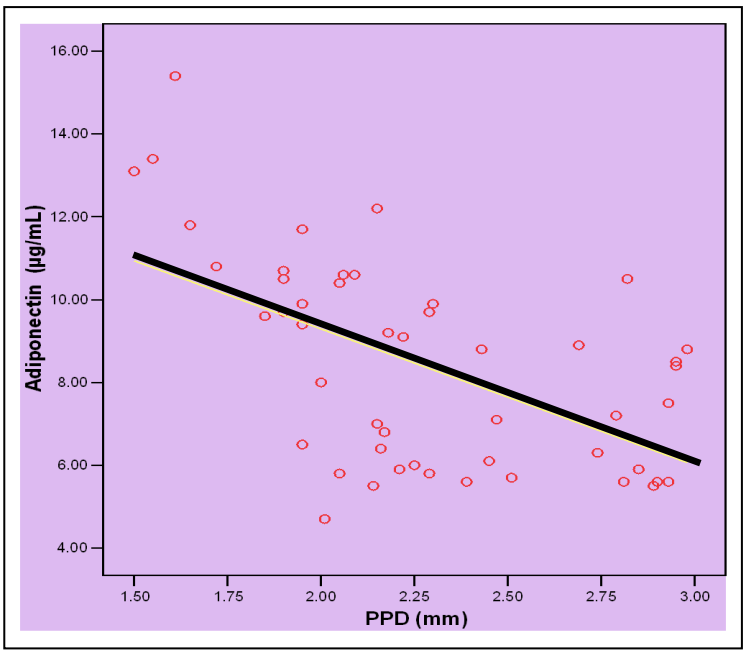


Figure 14: Correlation of CAL and Adiponectin level in Study group-I

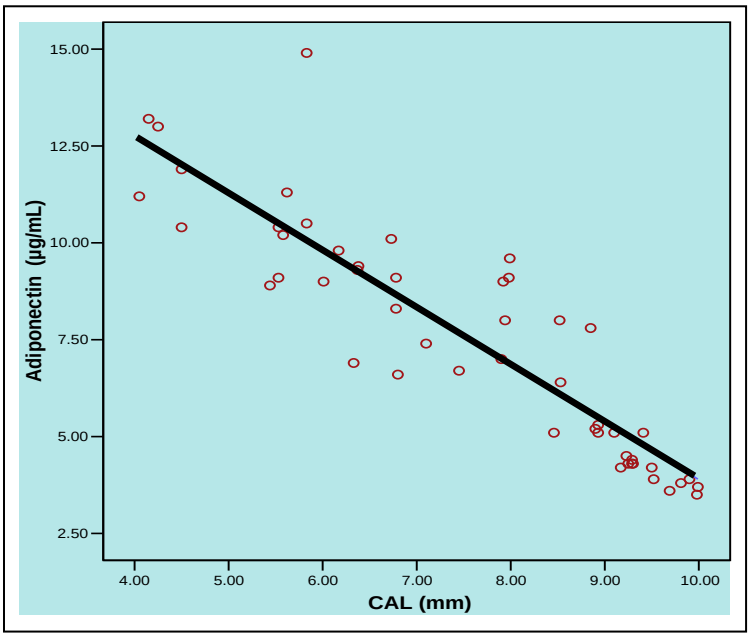


Figure 15: Correlation of CAL and Adiponectin level in Study group-II

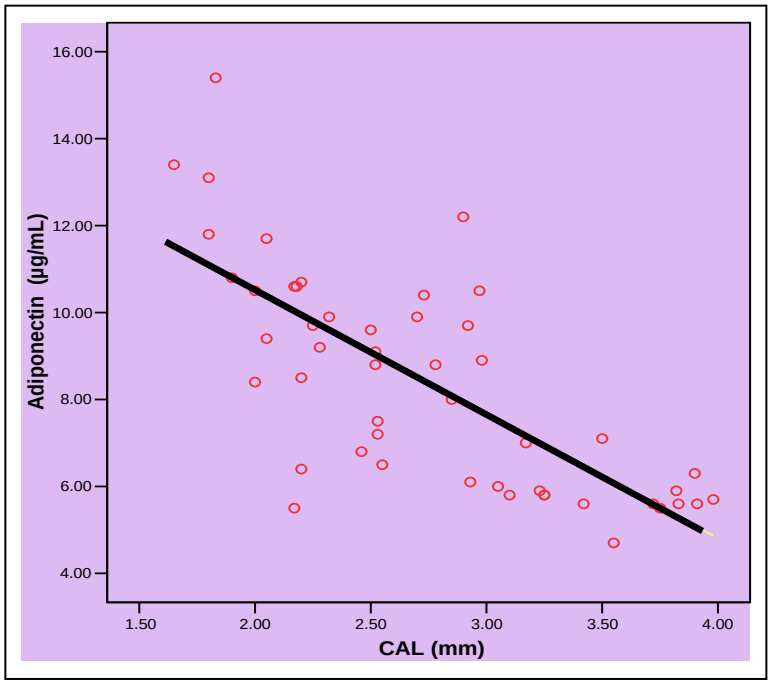


Figure 16: Correlation of FBS and Adiponectin level in Study group-I

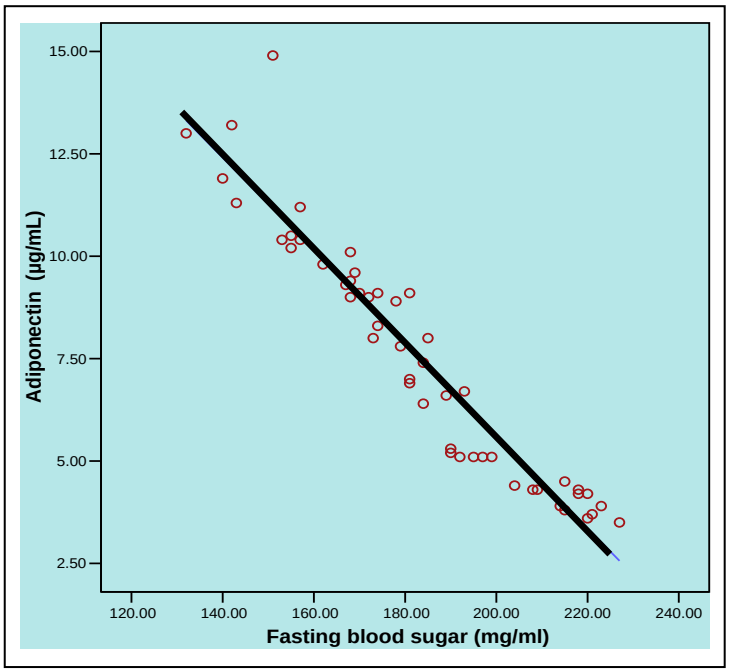
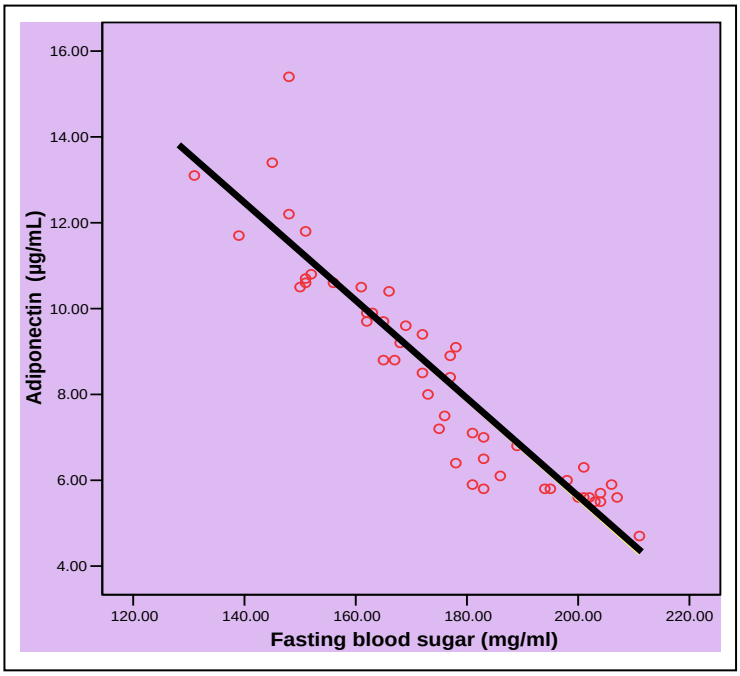


Figure 17: Correlation of FBS and Adiponectin level in Study group-II



DISCUSSION

The influence of Diabetes mellitus (DM) on periodontal disease has been discussed in the literature and also there is a substantial evidence to indicate that DM is a risk factor for periodontal disease. Conversely the effect of periodontal disease on glycemic control of diabetes has also been evaluated in many studies.

In this study we have evaluated the effect of non surgical periodontal therapy (NSPT) on clinical periodontal parameters, fasting blood sugar (FBS) and serum adiponectin level in type-2 diabetes mellitus (T2DM) patients with moderate to severe chronic periodontitis.

The mean FBS in healthy control is 94.5±9.176 mg/mL and the mean serum adiponectin level is 15.026±7.926 µg/mL.

In the present study, non-surgical therapy was performed under local anesthesia by using ultrasonic devices and hand instruments which is consistent with **Badersten et al**[5] who used hand instruments**.**

The main finding of this study was the satisfactory clinical response to non surgical therapy, which was followed by a reduction in clinical parameters, FBS level and an increase in serum adiponectin level. Hence there was a linear negative correlation found between clinical periodontal parameters, FBS and serum adiponectin level.

After initiation of the periodontal disease by putative subgingival pathogens, pro-inflammatory cytokines like TNF-α, IL-1 and IL-6 are produced in the local tissue as well as its levels are elevated in the circulation[45, 35]. TNF-α is an important inflammatory cytokine, closely linked to insulin resistance[11] which regulates CRP expression. Studies showed that circulating TNF-α and CRP levels are increased in T2DM patients with chronic periodontitis[45, 35].

In this present study, the mean PI value was 2.494 ± 0.147 in study group-I. There was a highly significant (p=0.000) reduction in mean PI value in study group-II (0.987± 0.079) when compared to study group-I. In study group-I the mean value of BOP was 90.407% ±5.385%, it was highly significantly (p=0.000) reduced to 7.561% ±2.289% in study group-II. Similarly, the other clinical parameters, the mean PPD and CAL value respectively in study group-I were 7.13±1.738 mm and 7.54±1.804 mm which was highly significantly (p=0.000) reduced respectively to 2.283±0.423 mm and 2.740±0.655 mm in study group-II. All these findings were in line with **Correa FOB et al[8] and Kiran et al[23]** study. This significant reduction between study group-I and II is due to pro-inflammatory cytokines in the periodontal tissue as mentioned above.

In this study, the non surgical therapy was performed without changing the anti-diabetic treatment including drugs and diet in study group-I subjects. Fasting blood sugar level was used as a measure of glycemic control, the mean FBS level in study group-I was 183.20±24.982 mg/mL when compared to group-II which was highly significantly (p=0.000) reduced to 175.20±20.360 mg/mL. This finding is in accordance with **Wei-Lian Sun et al[50]** who reported that moderate to poorly controlled T2DM patients with chronic periodontitis after periodontal intervention exhibited a significant reduction in FBS level.

Previous studies mentioned conflicting results on whether the non surgical therapy has impact on the glycemic control in T2DM. In the present study, we observed PPD, CAL and FBS level were significantly decreased in the study group-II as compared to study group I. These results confirmed that the non surgical therapy reduces clinically evident inflammation thereby reducing the insulin resistance[11] and improving glycemic control in T2DM. These findings indicate that inflammation is

involved in the pathogenesis of insulin resistance (IR) and T2DM, which is regarded as main processes in the mechanism of T2DM[4].

Adiponectin is the only adipocytokine exclusively secreted by adipose tissue, which profoundly influences insulin sensitivity by inhibiting glucose uptake by adipose tissue and muscle[52]. In the present study there was a highly significant (p=0.000) increase in adiponectin level 8.39±2.52µg/mL in study group-II, when compared to study group-I who had 7.52±2.96µg/mL. This finding is in concurrence with **Pariksha Bharti et al[36] and Kardesler et al[22],** who had also found elevation of serum adiponectin level after periodontal treatment. Another study by **Hotta et al[16]**, reported that type-2 diabetic patients had relatively lower serum adiponectin concentrations compared with non-diabetic patients which is similar to this study because we have found lower serum adiponectin level in T2DM (study group-I) patients compared with healthy controls who had 15.026±7.926 µg/mL.

We hypothesized that the non surgical therapy improves insulin sensitivity due to the elevation of adiponectin levels[52, 39]. The present study showed that there was an elevation of serum adiponectin level and a negative correlation found between the serum adiponectin level, clinical periodontal parameters and FBS level.

According to the present study there was a highly significant (p=0.000) elevation of serum adiponectin level in study group-II compared to study group-I. This is in partial accordance with **Matsumoto et al[31]**, who investigated the effects of anti microbial periodontal treatment (APT) on serum adiponectin level in T2DM patients with chronic periodontitis. Their results showed improvement periodontal conditions and increased serum adiponectin level, 2 months after periodontal treatment.

Hence, periodontal intervention helps in improving the glycemic control and insulin sensitivity which may be associated with decreased serum inflammatory cytokines and increased serum adiponectin level. The underlying mechanism of these inflammatory cytokines still remains unclear. However, **Nishimura et al**[35] found that chronic periodontal inflammation can lead to increased serum levels of TNF-α, thus inducing the phosphorylation of serine residues in the insulin receptor substrate-1, promoting the target cells to produce insulin resistance also acting on the liver to increase CRP synthesis. Many investigators suggested that TNF-α and other inflammatory cytokines may activate the intercellular pathways such as the I-kappa-B (IκB), I-kappa-B kinase (IκKB), nuclear factor-kappa B (NF-κB) and the protein c-Jun N-terminal kinase (JNK) axes, amplify and aggravate low-grade inflammation. These processes may become self-perpetuating through a positive feedback loop created by the pro-inflammatory cytokines and lead to insulin resistance and diabetes.

The mechanism for increased adiponectin could be through reduction of bacterial endotoxins (LPS) by periodontal treatment ameliorates the burden on immune system in adipose tissues, which led to the elevation of serum adiponectin levels[36]. Toll-like receptor ligands causes pro-inflammatory and pro-diabetic activation of adipocytes via c-Jun N-terminal Kinase (JNK)[24] and selective inactivation of JNK in adipose tissue-specific over expression of dominant-negative JNK transgenic mice[54]. These results suggested that the periodontal treatment increases serum adiponectin levels by reducing the influence of toll-like receptor ligands (such as LPS) from the inflamed periodontal tissue.

In the present study, there was statistically highly significant increase in serum adiponectin level in type-2 diabetes mellitus patients with moderate to severe chronic periodontitis 3 months after the non surgical therapy (study group II) as compared to

base line (study group I). There was highly significant decrease in fasting blood glucose level and periodontal clinical parameters observed 3 months after therapy. Therefore the effect of non surgical therapy on serum level of adiponectin is predictable and significant association was observed in type-2 diabetes mellitus with periodontal inflammation.

SUMMARY AND CONCLUSION

In the present study totally hundred subjects attending the Department of Periodontics were recruited. Out of the hundred subjects, fifty age and gender matched subjects with healthy periodontium were the control group, fifty type-2 diabetes mellitus patients with moderate to severe chronic periodontitis were the study group.

All the subjects have been examined to assess periodontal clinical parameters such as Plaque index, Bleeding on probing, Pocket probing depth and Clinical attachment level and were recorded. Fasting blood sugar was evaluated using glucometer for all the subjects and recorded. Venous blood was drawn for all hundred subjects and serum adiponectin level was analysed using ELISA method. Fifty type-2 diabetic with chronic periodontitis patients underwent non-surgical periodontal therapy using ultra-sonic scalers and hand instruments and put on maintenance for three months. After three months, periodontal status was reevaluated. Fasting blood sugar was estimated using glucometer and serum adiponectin level was analysed using ELISA method.

The findings are summarized as follows

1. In the healthy age and gender matched control group with healthy periodontium, the serum adiponectin level was 15.02±7.926µg/mL.

2. In type-2 diabetes mellitus with chronic periodontitis patients before non-surgical therapy (Study group-I) had decreased serum adiponectin level and the mean value being 7.52±2.96µg/mL.

3. In type-2 diabetes mellitus with chronic periodontitis patients three months after non-surgical therapy (Study group-II) had improvement in periodontal clinical parameters and a highly significant (p=0.000) increase in serum adiponectin level which was 8.39±2.52 µg/mL.

We would like to hypothesize that in this present study, non-surgical periodontal therapy improves periodontal status thereby increases serum adiponectin level in type-2 diabetes mellitus patients with moderate to severe chronic periodontitis.

BIBLIOGRAPHY

1. Ainamo J, Bay I. Problems and proposals for recording gingivitis and plaque. *Int. Dent. J* 1975; **25**: 229-235.
2. Armitage GC: Development of a classification system for periodontal diseases and conditions. *Ann Periodotol* 1999; **4:**1.
3. Arita Y, Kihara S, Ouchi N, et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun* 1999; **257**: 79-83.
4. Arner P. Insulin resistance in type-2 diabetes-role of the adiponectin. *Curr Mol Med* 2005; **5:** 333-339.
5. Badersten A, Nilveus R, Egelberg J. Effect of non surgical periodontal therapy in moderately advance periodontitis. *J Clin Periodontol* 1981; **8**: 57-72.
6. Carranza AF, Newman MG, Takei H Klokkevold PR. *Clinical Periodontology* ***10**th edition*, 552-553.
7. Choi BH, Kim YH, Ahn IS, Ha JH, Byun JM, Do MS. "The inhibition of inflammatory molecule expression on 3T3-L1 adipocytes by berberine is not mediated by leptin signaling". *Nutr Res Pract* 2009; **3 (2):** 84–8.
8. Correa FO, Gonclaves D, Figueredo CM, Gustafsson A, Orrico SR. Effect of periodontal treatment on metabolic control, systemic inflammation and cytokines in patients with type-2 diabetes. *J Clin Periodontol* 2010 Jan; **37(1):** 53-8.
9. Débora C. Rodrigues Dr. Mário Taba Jr.Arthur B. Novaes Jr.Sérgio L.S. Souza Márcio F.M. Grisi et al.Effect of Non-Surgical Periodontal Therapy on Glycemic Control in Patients with Type 2 Diabetes Mellitus. *J Periodontol*. 2003 Sep; **74(9):** 1361-7.

10. Dominik Kraus, Jochen Winter, Sobren Jepsen, Rainer Meyer, James Deschner et al. Interactions of adiponectin and Lipopolysaccharide from Porphyromonas gingivalis on Human Oral Epithelial Cells. PLoS One.2012; **7(2):** e30716
11. Engebretson S, Chertog R, Nichols A, Hey-Hadavi J, Celenti R, Grbic J. Plasma levels of tumor necrosis factor-α in patients with chronic periodontitis and type-2 diabetes. *J Clin Periodontol* 2007; **34**: 18-24.
12. Furugen R, Hayashida H, Yamaguchi N, Yoshihara A, Ogawa H, Miyazaki H, et al. The relationship between periodontal condition and serum levels of resistin and adiponectin in elderly Japanese. *J Periodontal Res* 2008; **43(5):** 556-62.
13. Grant DA, Irving B, Stern, Listgarten MA. *Periodontics* ***6**th* *edition*, 525-527.
14. Grimshaw CE, Matthews DA, Varughese KI, Skinner M, Xuong NH, Bray T, Hoch J, Whiteley JM). "Characterization and nucleotide binding properties of a mutant dihydropteridine reductase containing an aspartate 37-isoleucine replacement". *J. Biol. Chem.* August 1992; 267 **(22)**: 15334–9.
15. He W, Barak Y, Hevener A, et al, 2003 Adipose-specific peroxisome proliferator-activated receptor gamma knockout causes insulin resistance in fat and liver but not in muscle. *Proc Natl Acad Sci 2003;* **100**: 15712-15717.
16. Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y et al. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type-2 diabetic patients. *Arterioscler Thromb Vasc Biol* 2000; **20(6)**: 1595-9.
17. Hu E, Liang P, Spiegelman BM. AdipoQ is a novel adipose-specific gene dysregulated in obesity. *J Biol Chem* 1996; **271**: 10697-10703.
18. Hujoel PP, White Ba, Garcia RI, Listgarten MA. The dentogingival epithelial surface area revieited. *J Periodontal Res 2001;* **36**: 48-55, 2001.

19. Imagawa A, Funahashi T, Nakamura T, et al. Elevated serum concentration of adipose-derived factor,adiponectin, in patients with type 1 diabetes. *Diabetes Care* 2002; **25**: 1665-1666.
20. Ioanna Xynogala, Eudoxie Pepelassi, Deopina Perrea, George agrogiannis, Alkistis Pantopoula, Ioannis Vrotsos et al. Adiponectin and Interleukin-6 in nsulin-treated diabetic rats with experimental periodontitis. *Bras Oral res 2012;* Vol.**26**: no.1.
21. Kadowaki T, Yamauchi T, Kubotal N, Hara K, Ueki K, Tobe K. Adiponectin and adiponectin receptors in insulin resistance, diabetes and the metabolic syndrome. *J Clin Invest* 2006; **116**: 1784-1792,
22. Kardesler L, Buduneli N, Cetinkalp S, Kinane DF. Adipokines and inflammatory mediators after initial periodontal treatment in patients with type-2 diabetes and chronic periodontitis. *J Periodontol.* 2010 Jan; **81(1)**: 24-33.
23. Kiran M, Arpak N, Unsal E, Erdogan MF. The effect of improved periodontal health on metabolic control in type-2 diabetes mellitus. *J Clin Periodontol* 2005; **32**: 266-272.
24. Kopp A, Buechler C, Bala M, Neumeier M, Schaffler A. Toll-like receptor ligands cause proinflammatory and prodiabetic activation of adipocytes via phosphorylation of extracellular signal-regulated kinase and c-Jun N-terminal kinase but not interferon regulatory factor-3. *Endocrinology* 2010; **151(3)**: 1097-108.
25. Lei Chen, Bihon Wei, Jing Liu, Dongying Xuan, Baoyi Xie and Jincai Zhang. Association of periodontal parameters with metabolic level and systemic inflammatory markers in patients with type-2 diabetes. *J. Periodontology* March.2010; **81(3)**: Pages 364-371

26. Lodish HF, Bogan JS. Two compartments for insulin-stimulated exocytosis in 3T3-L1 adipocytes defined by endogenous ACRP30 and GLUT4. *The Journal of Cell Biology 1995;* **146(3)**: 609-20.
27. Loe H. Periodontal disease: The sixth complication of diabetes mellitus. *Diabetes Care* 1993; **16**: 329-334.
28. Loesche WJ, Lopatin DE. Interactions between periodontal disease, medical diseases and immunity in the older individual. *Periodontol 2000;* **16**: 80-105, 1998.
29. Lu HL, Wang HW, Wen Y, Zhang MX, Lin HH. Roles of adipocyte derived hormone adiponectin and resistin in insulin resistance of type-2 diabetes. *Worl J Gastroenterol* 2006; **12(11)**: 1747-51.
30. Maeda N, Shimomura I, Kishida K, Nishimura H, Matsuda M et al. Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. *Nat Med* 2002; **8(7)**: 731-7.
31. Matsumoto S, Ogawa H, Soda S, Hirayama S, Amarasena N, Aizawa Y, et al. Effect of antimicrobial periodontal treatment and maintenance on serum adiponectin in type- diabetes mellitus. *J Clin Periodontaol* 2009; **36(2):** 142-8.
32. Mealey BL, Rose LF. Diabetes mellitus and inflammatory periodontal diseases. Current opinion in endocrinology. *Curr Opin Edocrinol Diabetes Obes* 2008; **15**: 135-141.
33. Miller LS, Manwell MA, Newbold D, Reding ME, Rasheed A, Blodgett J, et al. The relationship between reduction in periodontal infl ammation and diabetes control: a report of 9 cases. *J Periodontol.* 1992 Oct; **63(10)**:843-8.
34. Nedvídkova J, Smitka K, Kopský V, Hainer V. "Adiponectin, an adipocyte-derived protein". *Physiol Res* 2005; **54 (2**): 133–40.

35. Nishimura F, Soga Y, Iwamoto Y, Kudo C, Murayama Y. P Periodontal disease as part of the insulin resistance syndrome in diabetic patients. *J Int Acad Periodontol* 2005; **7:** 16-20,

36. Pariksha Bharti, Sayaka Katagiri, Hiroshi Nitta, Toshiyuki Nagasawa, Hiroaki Kobayashi et al. Periodontal treatment with topical antibiotics improves glycemic control in association with elevated serum adiponectin in patients with type-2 diabetes mellitus. *Obes Res Clin Pract* 30 August 2011; **222**.

37. Pociot F, Briant L, Jon geneel CV, Molvig J, Worsaae H, AbbalM, et al Association of tumor necrosis factor (TNF) and classII major histocompatibility complex alleles with the secretionof TNF-alpha and TNF-beta by human mononuclear cells: apossible link to insulin-dependent diabetes mellitus. *Eur J Immunol*. 1993 Jan; **23(1):**224-31.

38. Qi C, Pekala PH. Tumor necrosis factor-alpha-inducedinsulin resistance in adipocytes. *Proc Soc Exp Biol Med.* 2000Feb; **223(2)**:128-35.

39. Reiko Furugen, Hideaki Hayashida, Masayasu Kitamura, Toshiyuki Saito Relationship between adipokines and periodontitis. Department of Oral Health, *Nagasaki University Graduate school of Biomedical sciences 2010;* 1-7-1:852-858.

40. Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF. A novel serum protein similar to C1q, produced exclusively in adipocytes. *J.Biol.chem* 1995; **270**: 26746-26749.

41. Shapiro L, Scherer PE. "The crystal structure of a complement-1q family protein suggests an evolutionary link to tumor necrosis factor". *Curr. Bio*. March 1998; **8 (6)**: 335–8.

42. Silness J, Loe J: Periodontal disease in pregnancy. II correlation between oral hygiene and periodontal condition. *Acta Odontologica Scandinavia* 1964; **22**: 112-135.

43. Smyth S, Heron A. Diabetes and obesity: The twin epidemics. *Nat Med* 2006; **12**: 75-80.

44. Stewart JE, Wager KA, F riedlander AH, Zadeh HH. The effect of periodontal treatment on glycemic control in patients with type 2 diabetes mellitus. *J Clin Periodontol Apr* 2001; **28(4):**306-10.

45. Sun WL, Chen LL, Zhang SZ, Ren YZ, Qin GM. Changes of adiponectin and inflammatory cytokines after periodontal intervention in type-2 diabetes patients and periodontitis. *Arch Oral Biol* 2010; **55:** 970-974.

46. Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS. Protection from obesity-induced insulin resistance in mice lacking TNF-α function. *Nature* 1997; **389**(6651): 610-4.

47. Waki H, Yamauchi T, Kamon J, Ito Y, Uchida S, Kita S, Hara K, Hada Y, Vasseur F, Froguel P, Kimura S, Nagai R. Impaired multimerization of human adiponectin mutants associated with diabetes. Molecular structure and multimer formation of adiponectin. *J. Biol. Chem* 2003; **278**: 40352.

48. Wang H, Zhang H, Jia Y, Zhang Z, Craig R, Wang X. & Elbein, S. C. Adiponectin receptor 1 gene (ADIPOR1) as a candidate for type 2 diabetes and insulin resistance. *Diabetes* 2004; **53:** 2132–2136.

49. Weidman F, Lalla E, Schnidt Am et al. Advanced glycation endproducts (AGEs) induce oxidant stress in the gingival: a potential mechanism underlying accelcrated periodontal disease associated with diabetes. *J Periodontal Res* 1996; **31(7)**: 508-515.

50. Wei-Lian Sun, Chen LL, Zhang SZ, Wu YM, REn YZ, Qin GM. Inflammatory cytokines, adiponectin, insulin resistance and metabolic control after periodontal intervention in patients with type-2 diabetes and chronic periodontitis. *Intern Med*. 2011; **50(15)**: 1569-74.

51. Yamauchi T, Kamon J, Ito Y, et al. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature* 2003; **423**: 762-769.

52. Yamauchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S et al. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med* 2002; **8(11)**: 1288-95.

53. Yamaguchi N, Kukita T, Li YJ, Martinez Argueta JG, Saito T, Hanazawa S, Yamashita Y. Adiponectin inhibits osteoclast formation stimulated by lipopolysaccharide from Actinobacillus actinomycetemcomitans. *FEMS Immunol. Med. Microbiol* 2007; **49**: 28-34.

54. Zhang X, Xu A, Chung SK, Cresser JH, Sweeney G, Wong RL, et al. Selective inactivation of c-Jun Nh2-terminal kinase in adipose tissue protects against diet-induced obesity and improves insulin sensitivity in both liver and skeletal muscle in mice. *Diabetes* 2011; **60(2)**: 486-95.

PROFORMA

Date:	Dental OP No:	Code No:
Name:	Age/ Sex:	Occupation:
Address:	Income:	Tel. No:

CHIEF COMPLAINTS

Pain/Shaky teeth/ Bleeding gums/ swollen Gums/ Receding Gums/ Pus Discharge/ Increase in Spacing between teeth/ stains/ others.

Duration:

Medical History:

Any relevant Medical History.

Family History:

Any familial relevant Medical History.

Dental History:

Periodontal treatment within past 6 months.

Personal History:

History of Smoking, Consumption of Alcohol

PLAQUE INDEX – SILNESS & LOE (1964)

18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38

Calculation:

Inference:

GINGIVAL BLEEDING INDEX – AINAMO & BAY (1975)

18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38

Inference

PROBING DEPTH (PD) & CLINICAL ATTACHMENT LEVEL (CAL) (in mm)

Maxillary

Palatal

CAL															
PPD															
	18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
PPD															
CAL															

Buccal

MANDIBULAR

Lingual

CAL																
PPD																
	48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38
PPD																
CAL																

Buccal

INVESTIGATION:

OPG

Complete Blood Count: Hb%, CT, BT, TC, DC

DIAGNOSIS:

Laboratory Analysis

RESULT: Date: Time:

1. Fasting Blood Sugar: (Reference Value: 80-110mg/dl)
2. Adiponectin : (Reference Value: 5-30mcg/ml)

AFTER PHASE-I THERAPY

GINGIVAL BLEEDING INDEX – AINAMO & BAY (1975)

18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38

Inference

PROBING DEPTH (PD) & CLINICAL ATTACHMENT LEVEL (CAL)
(in mm)

Maxillary

Palatal

CAL																
PPD																
	18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
PPD																
CAL																

Buccal

MANDIBULAR

Lingual

CAL																
PPD																
	48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38
PPD																
CAL																

Buccal

Laboratory Analysis

RESULT: Date: Time:

1. Fasting Blood Sugar: (Reference Value: 80-110mg/dl)
2. Adiponectin: (Reference Value: 5-30mcg/ml)

Signature of the PG student **Signature of the Guide**

Date :
Time :

INFORMED CONSENT FORM

Study Title

EVALUATION OF SERUM ADIPONECTIN LEVELS IN TYPE-II DIABETES MELLITUS WITH CHRONIC PERIODONTITIS- AN INTERVENTIONAL STUDY

Name: O.P.No:
Address: Code No:
Age/ Sex:
Tel. No:

I, _____ age _____ years exercising my free power of choice, hereby give my consent to be included as a participant in the study **"Evaluation of serum adiponectin levels in type-ii diabetes mellitus with chronic periodontitis- an interventional study"**

I agree to the following:

- I have been informed to my satisfaction about the purpose of the study and study procedures including investigations to monitor and safeguard my body function.
- I understand that the lab investigations will require the procurement of my blood in required amount.
- I agree to cooperate fully and to inform my doctor immediately if I suffer any unusual symptom.
- I have informed the doctor about all medications I have taken in the recent past and those I am currently taking.
- I hereby give permission to use my medical records for research purpose. I am told that the investigating doctor and institution will keep my identity confidential.

Name of the Patient **Signature/ Thumb Impression**

Name of the Investigator **Signature** **Date**

ஆராய்ச்சி ஒப்புதல் கடிதம்

நாட்பட்ட ஈறு அழற்சி நோய் மற்றும் பிரிவு - II சர்க்கரை நோயினால் பாதிக்கப்பட்டவர்களின் குருதி ஊரில் உள்ள அடிப்போநெக்டின் அளவினை கண்டறிதல்

பெயர்	:	தேதி	:
வயது	:	புற நோயாளி எண்	:
பாலினம்	:	ஆராய்ச்சி சேர்க்கை எண்	:

என்னுடைய சுய நினைவுடனும் மற்றும் முழு சுதந்திரத்துடன் இந்த மருத்துவ ஆராய்ச்சியில் சேர்த்துக்கொள்ள ஒப்புதல் அளிக்கிறேன்.

கீழ்காணப்படும் நிபந்தனைகளுக்கு நான் ஒப்புதல் அளிக்கிறேன்.

- இந்த ஆராய்ச்சியின் நோக்கமும், செயல் முறைகளும் எனக்கு திருப்தியளிக்கும் வகையில் அறிவுறுத்தப்பட்டது.
- இந்த பரிசோதனை செய்வதற்காக எனது குருதியை பரிசோதனைக்கு எடுக்க வேண்டுமென்று அறிகிறேன்.
- நான் ஏற்கனவே உட்கொண்ட மற்றும் உட்கொள்கிற மருந்துகளைப் பற்றிய விபரங்கள் ஆராய்ச்சியாளரிடம் தெரிவித்துள்ளேன்.
- என் உடல்நலம் பாதிக்கப்பட்டாலோ அல்லது எதிர்பாராத வழக்கத்திற்கு மாறான நோய்க்குறி தென்பட்டாலோ அதனை உடனடியாக மருத்துவரிடம் தெரிவிக்க சம்மதிக்கிறேன்.
- என் மருத்துவ குறிப்பேடுகளை இந்த ஆராய்ச்சியில் பயன்படுத்திக்கொள்ள சம்மதிக்கிறேன். இந்த ஆராய்ச்சி மையமும், ஆராய்ச்சியாளரும் என்னுடைய விவரங்கள் அனைத்தையும் இரகசியமாக வைப்பதாக அறிகிறேன்.

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நோயாளியின் பெயர்	கையொப்பம்	தேதி

.....	.....	.....
ஆராய்ச்சியாளரின் பெயர்	கையொப்பம்	தேதி

INFORMATION SHEET

- We are conducting a study on "**Evaluation of Serum Adiponectin levels in Type-II Diabetes Mellitus with chronic Periodontitis – An Interventional Study**" among patients attending TNGDCH, Chennai and for this study, we are selecting patients.

- The identity of the patients participating in the research will be kept confidential throughout the study. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared

- Taking part in this study is voluntary. You are free to decide whether to participate in this study or to withdraw at any time; your decision will not result in any loss of benefits to which you are otherwise entitled.

- The results of the special study may be intimated to you at the end of the study period or during the study if anything is found abnormal which may aid in the management or treatment.

Name of the patient **Signature/Thumb impression**

Name of the investigator **Signature** **Date**